

Residues of some veterinary drugs in animals and foods

FAO
FOOD AND
NUTRITION
PAPER

41/7

***Azaperone
Carazolol
Dexamethasone
Dihydrostreptomycin
and streptomycin
Enrofloxacin
Gentamicin
Neomycin
Oxolinic acid
Spiramycin***

WORLD
HEALTH
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Food
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Nations



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Monographs prepared by the
forty-third meeting of the
Joint FAO/WHO Expert Committee
on Food Additives

Geneva, 15-24 November 1994

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Geneva, 15-24 November 1994

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ABBREVIATIONS USED IN THIS DOCUMENTS

ADI	-acceptable daily intake	μm	-micrometer
AUC	-area under concentration-time curve	mg	-milligram
b.i.d.	-twice a day	min	-minute
BP	-British Pharmacopoeia	ml	-millilitre
Bq	-Becquerel (one disint/sec)	MR	-marker residue
BST	-bovine somatotropin	MRL	-maximum residue limit
bw, BW	-body weight	MS	-mass spectrometry
$^{\circ}\text{C}$	-degrees Celcius	n or No	-number
^{14}C	-radioactive Carbon-14	na	-not analyzed, assayed or available
C_{max}	-maximum concentration	nd	-not detected
CAP	-chloramphenicol	NER	-non extractable residues
μCi	-microcuries of radioactivity	ng	-nanogram
cm^3	-cubic centimeter	nm	-not measured, if applicable
conc	-concentration	nm	-nanometer, if applicable
CV	-coefficient of variation	NMR	-nuclear magnetic resonance
d	-day	NOEL	-no-observed-effect level
DPM	-disintegration per minute	ppb	-parts per billion
e.g.	-for example	ppm	-parts per million
EP	-European Pharmacopoeia	r	-regression coefficient
eq or EQ	-equivalents	RIA	-radioimmunoassay
F	-female	SA	-Specific Activity
g	-gram	s.c.	-subcutaneous
μg	-microgram	SD	-standard deviation
GC	-gas chromatography	s.i.d.	-once a day
GI	-gastrointestinal	$t_{1/2}$	-half life
GLC	-gas-liquid chromatography	t_{max} or T_{max}	-time for maximum
GLP	-Good Laboratory Practice	TLC	-thin layer chromatography
GVP	-Good Veterinary Practice	TMS	-trimethyl silyl
h	-hour	TR	-total residues
^3H	-tritium	TRA	-total radioactivity
HPLC	-high performance liquid chromatography	TSD	-termionic specific detection
i.e.	-that is	UD	-unchanged drug
i.m., IM	-intra muscular	USDA	-US Department of Agriculture
i.m.i.	-intra muscular injection	USP	-United States Pharmacopoeia
i.p.	-intra peritoneal	UV	-ultraviolet
i.v., IV	-intra venous	V_d	-volume of distribution
kg	-kilogram	v/v	-volume/volume
L or l	-litre	wt	-weight
LC	-liquid chromatography	w/v	-weight/volume
LOD	-limit of detection	WT	-withdrawal time
LOQ	-limit of quantitation	%	-per cent
M	-molar or mole	>	-greater than
M	-male	<	-less than
max	-maximum	$\leq$	-equal or less than

INTRODUCTION

The Monographs on the residues of the ten compounds contained in this volume were prepared by the forty-third meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), which was held in Geneva, 15-24 November 1994. JECFA has evaluated veterinary drugs at previous meetings, including the 12th¹, 26th², 27th³, 32nd⁴, 34th⁵, 36th⁶, 38th⁷, 40th⁸, and 42nd⁹ meetings. In response to a growing concern about mass-medication of food producing animals and the implications for human health and international trade, a Joint FAO/WHO Expert Consultation on Residues of Veterinary Drugs was convened in Rome, in November 1984¹⁰. Among the main recommendations of this consultation were the establishment of a specialized Codex Committee on Residues of Veterinary Drugs in Foods (CCRVDF) and the periodic convening of an appropriate body to provide independent scientific advice to this Committee and to the member countries of FAO and WHO. At its first session in Washington D.C. in November 1986, the newly-created CCRVDF reaffirmed the need for such a scientific body and made a number of recommendations and suggestions to be considered by JECFA¹¹. In response to these recommendations, the thirty-second JECFA meeting was entirely devoted to the evaluation of residues of veterinary drugs in foods. Subsequently, the 34th, 36th, 38th, 40th, 42nd, and 43rd meetings of JECFA were also devoted only to evaluation of veterinary drugs.

The eight session of the CCRVDF, held in Washington D.C. during June 1994, revised the priority list of veterinary drugs requiring evaluation. The drugs evaluated during the 43rd meeting of JECFA included these compounds.

The present volume contains summary monographs of the residue data on all of the ten compounds on the agenda. The β -adrenoceptor blocking agent, carazolol was considered for pigs. Carazolol had been previously evaluated by the 38th meeting of JECFA.

From the seven antimicrobial agents, dihydrostreptomycin, neomycin, spiramycin and streptomycin, had previously been evaluated by the Committee at the 12th meeting. From these spiramycin had been re-evaluated at the 38th meeting. The remaining antimicrobial agents, enrofloxacin, gentamicin and oxolinic acid, had not been evaluated before.

The tranquilizer, azaperone, previously evaluated at the 38th meeting of the Committee, was considered for pigs only.

The glucocorticosteroid, dexamethasone, had been evaluated before at the 42nd meeting and was considered for cattle and pigs. At this meeting dexamethasone was considered for residues in horses only.

The pertinent information in each monograph was discussed and appraised by the entire Committee. The monographs are presented in a uniform format covering identity, residues in food and their evaluation, metabolism studies, tissue residue depletion studies, methods of residue analysis and a final appraisal of the study results. More recent publications and documents are referenced, including those on which the monograph is based. A summary of the JECFA evaluations from the 32nd to the present 43rd meeting is included in Annex 1.

The assistance of the experts and FAO consultants in preparing these monographs is gratefully acknowledged.

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AZAPERONE

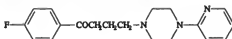
First draft prepared by
Dr. R.J. Heitzman
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IDENTITY

Chemical name: 1-(4-fluorophenyl)-4-[4-(2-pyridinyl)-1-piperazinyl]-1-butanone

Synonyms: CAS-1649-18-9, R-1929 (sponsor code)
Stresnil or Suicalm (Trademarks, Janssen)

Structural formula:



Molecular formula: $C_{19}H_{22}FN_2O$

Molecular weight: 327.40

OTHER INFORMATION ON IDENTITY AND PROPERTIES

Pure active ingredient: Azaperone (sum of impurities maximum 0.5 %)

Appearance: Almost white to slightly yellow powder

Melting Point: 92 - 95°C.

Solubility: Very slightly soluble in water (50 mg/l) and readily soluble in organic solvents especially, dichloromethane, N,N-dimethylformamide, tetrahydrofuran, toluene, 2-butanone, acetone and ethyl acetate.

Optical rotation: None

Ionization constant: $pK_{a2} = 4.3$, $pK_{a1} = 7.5$

UV_{max}: 243 nm and 312 nm.

RESIDUES IN FOOD AND THEIR EVALUATION

CONDITIONS OF USE

Azaperone is a butyrophenone derivative used as a neuroleptic sedative in pigs. The most commonly observed behavioral effect is the reduction of aggression.

Dosages

The dose to reduce aggressive behaviour is 2 mg/kg body weight (BW) intramuscularly (i.m.). The dose for the non-approved use to reduce transport stress prior to slaughter is as low as 0.4 mg/kg BW.

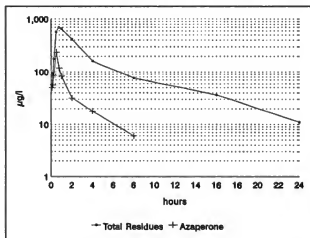
METABOLISM

Pharmacokinetics

Fig

One pig weighing 35 kg was injected i.m. with a single dose of 1 mg/kg BW of tritiated (^3H) azaperone (specific activity (SA) 13 mCi/mM). Blood, urine and faeces were collected over a 24 hour period after dosing and analyzed for total radioactive residues and additionally in the case of blood (plasma) the amount of unchanged drug was measured (method unclear)(K10). The results for blood are shown in Figure 1 and indicate a rapid absorption of the drug into the blood and reaching peak concentrations in about one hour. Thereafter there was a steady depletion with an elimination half-life after 4 hours of approximately 6 hours for the total residues and 2.5 hours for azaperone. At four hours more than 90% of the residue was metabolites of azaperone indicating rapid metabolism and degradation of the parent drug.

Figure 1. Plasma levels of total radioactivity and unchanged azaperone in one pig after i.m.i. of 1 mg/kg BW of tritiated azaperone.



About 89% of the total radioactivity was excreted in the urine during the first 24 hour after dosing, while less than 1% was found in the faeces.

In a second study (K19) pigs were dosed intramuscularly with ^3H -azaperone at 4 mg/kg BW. 60% of the radioactivity was excreted in the urine and 15% in the faeces in a 62 hour collection period.

In a new study (K22) using the recommended dose of 2 mg/kg BW, plasma levels of azaperone and azaperol were measured and shown to decline very rapidly (see below).

Rat

After subcutaneous injection of azaperone at 1 mg/kg BW 78.5% of the dose was excreted in the faeces and 22.3% in the urine during a four day period (K1). This is in marked contrast to the target species where the major route of excretion is the urine.

Metabolism in Food and Laboratory Animals

No new data was requested or submitted. The metabolism in the pig and the rat were fully reviewed at the 38th JECFA (FAO 41/4). In summary the data showed extensive metabolism in both species, using both *in vivo* and *in vitro* studies. The main metabolic pathways were:

- i) reduction of the butanone
- ii) hydroxylation of the pyridine group
- iii) oxidative N-dealkylation
- iv) oxidative dearylation.

The primary differences between what was observed in the pig compared to the rat were the large differences in the relative amounts of the various metabolites. The reductive pathway of the butyrophene predominated to a greater degree in the pig than in the rat. Also the reduced N-dearylated metabolite was found in much higher amounts in the pig than in the rat. Rat liver incubates had twice the amount of azaperol than pig liver homogenates.

Azaperone had 4-30 times the biological potency of azaperol in mice (Rauws et al., 1978). The activity of azaperol may be associated with its ready interconversion with azaperone. None of the other metabolites were shown to have significant neuroleptic activity (see V8672).

TISSUE RESIDUE DEPLETION STUDIES

Radiolabeled Residue Depletion Studies

The sponsors presented data at the 38th JECFA and no further data was requested. Two studies were performed in pigs and used doses of azaperone of either 1 mg/kg or 4 mg/kg BW. After administration of 1 mg/kg BW of ³H-azaperone intramuscularly to pigs the results are given in Table 1.

Table. 1. Total radioactivity (TRA) and unchanged drug (UD in pig tissues after administration of a 1 mg/kg BW dose of ³H-Azaperone given i.m.

Tissue	µg/kg wet tissue							
	4 hour		8 hour		16 hour		24 hour	
	TRA	UD	TRA	UD	TRA	UD	TRA	UD
Brain	107	12	91	13	29	nd	23	nd
Heart	87	nd	57	nd	12	nd	nd	nd
Lung	541	35	308	27	111	13	58	8
Kidney	1485	42	630	25	111	6	75	4
Liver	873	43	922	58	298	38	230	12
S-Intestine	168	19	118	21	37	nd	28	nd
L-Intestine	135	20	157	16	45	nd	20	nd
Muscle	69	15	40	nd	4	nd	nd	nd
Subcut Fat	282	60	117	40	68	nd	28	nd

The percentage of azaperone in kidney at 4, 8, 16, and 24 hours was 2.8%, 4.0%, 5.4% and 5.3%, respectively. The percentage of azaperone in liver at 4, 8, 16, and 24 hours was 4.9%, 6.3%, 12.8% and 5.2%, respectively.

The second study used eight pigs weighing 15-25 kg and were dosed intramuscularly with 4 mg/kg BW ³H-azaperone. The results are summarised in Table 2.

Table 2. Total radioactivity (TRA), unchanged drug (UD) and azaperol (*) in pig tissues after administration of a 4 mg/kg BW dose of ³H-Azaperone given i.m.

Tissue	$\mu\text{g/kg wet tissue}$							
	2 hour		24 hour		48 hour		72 hour	
	TRA	UD	TRA	UD	TRA	UD	TRA	UD
Kidney	11019	298 1290*	625	26 38*	204	14 13*	124	5 34*
Liver	3674	72 678*	698	23 56*	441	15 27*	228	11 9*
Muscle	588	44 258*	41	4 2*	20	2 0.8*	13	1.0 0.4*
Fat	1217 ^b	444 954*	166	70 58*	71	15 10*	104	13 6*
Skin	1324	151 523*	263 ^b	244 188*	64	15 12*	37	3 4*
Inj. Site	173900	131120 35300*	60420	53470 3080*	44380	35100 3500*	5805	4050 450*

* is azaperol; * one value unexpectedly high.

^b note this value is lower than sum of identified residues.

Other Residue Depletion Studies (with Unlabeled Drug)

Pigs

The sponsors have submitted a new study (K22) determining the magnitude and decline of residues of azaperone in the edible tissues (including the injection site) and plasma of pigs after dosing with 2 mg/kg BW intramuscularly. Twenty pigs weighing 79-96 kg were dosed and they were slaughtered in groups of four (2 males and 2 females) at 1, 2, 3, 5 and 7 days after injection and tissue and plasma samples collected and analyzed by HPLC/UV for residues of azaperone and azaperol. Plasma was also collected at 3 hours and 24 hours from all the pigs. Plasma levels of both azaperone and azaperol declined rapidly. Three hours after dosing the average level was 23 $\mu\text{g/l}$ for azaperone and 44 $\mu\text{g/l}$ for azaperol. From 24 hours onwards the plasma levels of both compounds were not detectable (LOD 5 $\mu\text{g/l}$) except for 2 animals in which the values at 24 hours were 13 and 21 $\mu\text{g/l}$ for azaperone and 9 $\mu\text{g/l}$ for azaperol. One animal out of four tested had a level of 7 $\mu\text{g/l}$ for azaperone at 48 hours.

The results for the residues in the edible tissues are summarised in Table 3. No residues were detected (LOD 25 $\mu\text{g/kg}$) at 5 days or 7 days after dosing. No residues of azaperone were detected in liver or kidney and no residues of azaperol were detected in muscle. In one pig muscle sample at day 2 residues of 76 $\mu\text{g/kg}$ for azaperone were detected. By day 3 the only detectable residues were azaperone in one fat sample and azaperol in one kidney sample.

Table 3. Residues ($\mu\text{g/kg}$) of azaperone (AZAP) and azaperol (AZOL) in tissues of pigs after intramuscular injection of azaperone at 2 mg/kg body weight.

	Day 1		Day 2		Day 3	
	AZAP	AZOL	AZAP	AZOL	AZAP	AZOL
Muscle	nd	nd	76	nd	nd	nd
Liver	nd	64, 71, 80	nd	36, 56	nd	nd
Kidney	nd	57, 64, 74, 63	nd	28, 28	nd	28
Fat	31, 41, 44	79, 75, 60, 29	26	34, 26	44	nd
Skin	60, 92	29, 36, 63, 66	nd	nd	nd	nd
Lard	26	26, 42	nd	nd	nd	nd

No residues were detected at day 5 or on day 7. nd means no residues were detected in all four pigs per collection time (LOD 25 $\mu\text{g/kg}$).

The injection site 317-510 g was excised and the residues of azaperone and azaperol measured. The results are shown in Table 4. There were significant residues remaining at the injection site for at least five days. By day 7 residues were detectable in only one of the four pigs. Azaperone appears to be partially metabolised to azaperol at the site of injection.

Bound Residues/Bioavailability

No data was submitted and no new data was requested.

METHODS OF ANALYSIS FOR RESIDUES IN TISSUES

The analytical methods available were fully reviewed at the 38th meeting of JECFA. They included methods for measuring both azaperone and azaperol by gas chromatography, thin layer chromatography and HPLC. It was concluded that the methods were sensitive enough for monitoring residues of interest and particularly for the determination of residues if the drug had been used immediately prior to the transport of pigs to slaughter.

The sponsors submitted new data (K34) showing that their HPLC method had been further developed and optimized to give better sensitivity and precision. Tissues (amount not given) were ground in a Waring blender and then homogenised in water. The chlorine analogue of azaperone was added as an internal standard before extraction with heptane-isoamyl alcohol (98.5:1.5, v/v). The extract was extracted into 0.1N sulphuric acid and back extracted at a high pH into the organic mixture. The extract was dried and dissolved in the HPLC mobile phase. HPLC was performed in the reverse phase with UV detection. Calibration curves were obtained for fortified tissues using azaperone and azaperol and a constant amount of internal standard. The concentrations in actual samples were obtained by interpolation on daily repeated calibration curves.

The recoveries of azaperone, azaperol and the internal standard were >90% for all tissues and plasma. The limit of quantification was 25 $\mu\text{g/kg}$ for tissues and 5 $\mu\text{g/l}$ for plasma. The accuracy in tissues ranged from 92 to 102% for azaperone and 92 to 109% for azaperol. The precision ranged from 1.7 to 6.5% for azaperone and 0.2 to 8.3% for azaperol. The residues were stable in deep frozen (-20°C) tissues and plasma for at least two months. [Note: There is no repeatability study for incurred material and the internal standard and spikes are added to the extract after homogenisation.]

Table 4. Residues of azaperone and azaperol at the injection sites of pigs dosed intramuscularly with azaperone at 2 mg/kg body weight.

	Day 1	Day 2	Day 3	Day 5	Day 7
Azaperone					
Conc., range (mg/kg)	6.96 - 51.9	2.29 - 71.8	0.16 - 11.1	4.23 - 29.6	<0.025 - 0.16
Mean (mg)	31.6	24.9	4.11	11.9	0.045
Total, range (mg/kg)	2.99 - 19.1	0.99 - 36.6	0.07 - 4.16	1.93 - 9.68	0.066 (1)
Mean (mg)	13.4	12.1	1.60	4.22	0.066
Azaperol					
Conc., range (mg/kg)	1.29 - 8.25	0.37 - 3.84	0.03 - 1.68	0.54 - 1.44	<0.025 - 0.045
Mean (mg)	5.17	1.84	0.62	1.00	0.03
Total, range (mg/kg)	0.56 - 3.86	0.16 - 1.96	0.01 - 0.63	0.17 - 0.48	0.019 (1)
Mean (mg)	2.21	0.86	0.24	0.38	0.019

The number in parenthesis indicates only one sample out of the four was positive.

Where no residue was detected the nominal value of the LOD was used for the calculation of the mean value.

APPRAISAL

Azaperone, a neuroleptic agent for use in pigs, was evaluated at the 38th JECFA. No ADI or MRL were recommended. JECFA requested "Studies on the concentrations of residues of azaperone and azaperol in both muscle and fat of pigs treated with azaperone over a three day period."

The sponsors have answered the request in full and also provided further information on residues in other edible tissues (see Table 3).

Pigs were injected intramuscularly with azaperone at the recommended dose of 2 mg/kg body weight (BW). They were slaughtered in groups of four at 1, 2, 3, 5 and 7 days after the injection. The residues in edible tissues of both azaperone and azaperol were measured by a new and improved method using HPLC with an LOD of 25 µg/kg. The results are shown in Table 3. No residues were detected in any tissue at 5 and 7 days. The only residues detected at day 3 were residues of azaperone in one pig fat sample and of azaperol in one kidney sample. In the muscle samples, 19 out of 20 pigs had no detectable residues and one sample showed a residue of azaperone (but not azaperol) on day 2. The results for fat indicated that residues were found in all four pigs on day 1, two pigs on day 2 and one pig on day 3. The concentration of the residues in all tissues did not exceed 100 µg/kg at any sampling time.

Maximum Residue Limits

As suggested at the 38th meeting both kidney and liver were possible marker tissues. There was great variation in the ratios of azaperone to azaperol in different tissues and at different sampling time points and this made it almost impossible to use one of the compounds as the sole marker of the total residues. A more reliable marker of total residues was obtained if the sum of both compounds were used. The ratio of the concentration of azaperone plus azaperol as a percentage of total residues was not known for the recommended dose (2 mg/kg BW), however, for the 4 mg/kg BW dose it was possible to calculate the ratio over a three day withdrawal time. The mean value for the percentage at all sampling times was 27% (range 6 - 115%) and at 72h after injection the mean average percentage was 11%.

The residues as azaperone equivalents which have neuroleptic activity have to be considered in the setting of MRLs. However only azaperone and azaperol are thought to possess significant neuroleptic potency and are approximately 10% of the total residues in tissues; the remaining 90% of residues can be discounted in calculating the MRLs.

The Committee recommends temporary MRLs of 60 µg/kg for muscle and fat and 100 µg/kg for liver and kidney

of pigs expressed as the sum of azaperone and azaperol. Using these values for the MRLs and taking into account the factors above, the ingested residues of parent drug and azaperol are muscle (300 g) 18 µg, liver (100 g) 10 µg, kidney (50 g) 5 µg and fat (50 g) 3 µg. The total is 36 µg/day.

The Committee noted that there were residues of azaperone and azaperol at the injection site and that their concentrations can exceed the MRL set for muscle tissue during a 7 day withdrawal period.

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- K34. V8143. Sponsor submission of Janssen. (1992). HPLC-UV method for the determination of azaperone and azaperol in animal tissues and plasma: validation and application to the azaperone residue study in pigs after intramuscular administration.
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- Rauws, A.G., Olling, J., Freudenthal, J. and Ten Ham, M. (1976). Azaperol, a new metabolite of the veterinary butyrophenone tranquilizer Azaperone. *Toxicol. Appl. Pharmacol.* **35**, 333-339. [Also submitted by sponsor as Report K11, V1782]

CARAZOLOL

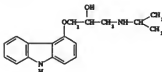
First draft prepared by
Dr. R. C. Livingston
Center for Veterinary Medicine
FDA, Rockville, USA

IDENTITY

Chemical names: 4-(2-hydroxy-3-isopropyl-amino-propoxy)carbazole;
1-(carbazol-4-yl-oxy)-2-hydroxy-3-isopropylaminopropane;
1-(4-carazolyloxy)-3-(isopropylamino)-2-propanol

Synonyms: Carazolol

Structural formula:



Molecular formula: $C_{18}H_{22}N_2O_2$

Molecular weight: 298.34

OTHER INFORMATION ON IDENTITY AND PROPERTIES

Pure active ingredient: Carazolol (98.0 to 102% by titrimetric assay with respect to dry substance)

Appearance: Pale yellow crystalline powder

Melting point: 135-138°C

Solubility: Water - practically insoluble
Chloroform - slightly soluble
Ethanol (96%) - soluble
Acetone - soluble
Methanol - very soluble

UV_{max}: 286 ± 1 nm

Storage: Should be stored in tightly closed containers free from light

RESIDUES IN FOOD AND THEIR EVALUATION

CONDITIONS OF USE

Carazolol is a β -blocking agent used as a tranquilliser to prevent stress in animals and humans. It is most widely used in pigs to ameliorate stress when they are transported, thus preventing losses due to death and deterioration of the meat quality. It is also used in female pigs to relieve the stress of parturition and in male pigs to placate and prevent frenzy during mating. A less widespread use is in the prevention of reproductive stresses in dairy cows. The drug may alleviate stress during calving and may also have a positive effect on conception rate.

The dose for pigs is 10 μg per kg BW administered as an intramuscular (IM) injection behind the ear. The dose used in cattle in the research studies was either 5 mg intravenously (IV) or 10 mg IM to cows weighing approximately 500 kg.

The full effects of the drug are obtained about 20-30 minutes after intramuscular injection and within 5 minutes after intravenous administration. The effects are maintained for 8-12 hours in the pig and for about 4 hours in cattle.

PREVIOUS REVIEWS

Carazolol was previously reviewed at the 38th JECFA meeting. The data reviewed included: a radiometric pharmacokinetics and excretion study in pigs; two pharmacokinetics studies in cattle; a metabolism study in pigs, dogs, and humans; two radiolabeled residue depletion studies in pigs; two residue depletion studies in pigs; a residue depletion study in cattle; and an HPLC method of analysis.

The Committee recommended temporary MRLs for carazolol of 5 $\mu\text{g}/\text{kg}$ for muscle and fat and 30 $\mu\text{g}/\text{kg}$ for liver and kidney in both cattle and pigs.

The Committee requested the results of the following residue studies for evaluation:

1. Radiometric studies on the concentrations of carazolol and its metabolites as proportions of the total residue in pigs and cattle over a 24-hour period.
2. Non-radiometric studies on carazolol residues in pigs, using suitable analytical methods, over a 24-hour period.

TISSUE RESIDUE DEPLETION STUDIES

Non-radiometric Residue Depletion Studies

Pig

Sixteen Landrace x Pietrain pigs (8/sex), weighing 60-80 kg, received 10 $\mu\text{g}/\text{kg}$ of carazolol by IM injection in the neck (Delahaut, 1994). The animals were slaughtered by groups of four at 2, 12, 18, and 24 hours after administration. Samples of muscle, kidney, fat, liver, skin, and tissue at injection site were taken. The residues of carazolol were determined by a validated HPLC method with fluorometric detection. The limit of quantification was 2.0 $\mu\text{g}/\text{kg}$. The limit of detection was approximately 0.2 $\mu\text{g}/\text{kg}$ for muscle, kidney, and skin; 0.7 $\mu\text{g}/\text{kg}$ for liver; and 0.6 $\mu\text{g}/\text{kg}$ for fat. The results are shown in Table 1.

Table 1. Residues of Carazolol in Pigs Using an HPLC Method ($\mu\text{g/kg}$; mean $\pm$ SEM)

Tissue	Withdrawal Time (hours)			
	2	12	18	24
Injection Site	56.8 $\pm$ 25.9	2.1 $\pm$ 2.8	6.5 $\pm$ 10.6	3.4 $\pm$ 6.4
Muscle	1.8 $\pm$ 0.5	<0.2	<0.2	<0.2
Kidney	6.9 $\pm$ 3.5	1.0 $\pm$ 0.4	0.4 $\pm$ 0.03	0.4 $\pm$ 0.03
Liver	9.9 $\pm$ 4.0	0.82 $\pm$ 0.6	0.2 $\pm$ 0.4	<0.7
Fat	1.8 $\pm$ 0.4	<0.6	<0.6	<0.6
Skin	2.5 $\pm$ 0.6	0.4 $\pm$ 0.1	0.4 $\pm$ 0.6	<0.2

The highest concentration of residues was found at the injection site and in the liver and kidney. The carazolol concentrations found in the different tissues in this study are of the same order of magnitude as previously reported.

Table 2 provides a comparison of the residues measured by HPLC in this study to those found in the previous radiolabeled studies at the 2-hour withdrawal time. The comparison was made only at the 2-hour withdrawal time point as it was the only withdrawal time that the two studies had in common.

Table 2. Comparison of ^{14}C -Carazolol Residues and Carazolol Residues Measured by HPLC ($\mu\text{g/kg}$)

Tissue	^{14}C -Carazolol	Carazolol (HPLC)	Ratio HPLC/ ^{14}C
Muscle	1.9	1.8	0.95
Liver	31.0	9.9	0.32
Kidney	19.2	6.9	0.36
Fat	4.0	1.8	0.45

Comparing these, it appears that the majority of residues in muscle tissue are parent drug. However, only approximately 30% of the residues are parent carazolol in liver and kidney.

Activity of metabolites of carazolol

The pharmacological activity of seven potential metabolites of carazolol was assessed in rabbits. The compounds examined were carazolol amine, carazolol lactate, carazolol diol, carazolol acetate, 4-hydroxylcarbazole, and carazolol glucuronide R(+) and S(-). Only the lactate, acetate and glucuronide have been identified as metabolites in the urine of pigs and humans. The other compounds were considered potential breakdown products of carazolol.

Each compound was infused into conscious rabbits over a period of 15 minutes. Twenty minutes after the end

of infusion, isoproterenol (1 µg/kg) was injected intravenously and the 30% and 50% inhibitory dose (ID) was determined. Dose-effect curves were constructed from 2-6 experiments with the given dose, performed on 8-10 rabbits. The results are given in Table 3.

Table 3. β -Receptor Blocking Activity of Intravenous Carazolol Metabolites Expressed as Inhibitory Dose (µg/kg)

Substance	No.	ID ₅₀	ID ₅₀
amine	6	22	53
lactate	2	>2000	>2000
diol	2	>2000	>2000
acetate	2	>6000	>6000
4-hydroxylcarbazole	2	>3000	>3000
R(+)-glucuronide	5	>3000	>3000
S(-)-glucuronide	2	2257	3585

Earlier work demonstrated that the ID₅₀ for IV carazolol is about 5 µg/kg.

The metabolites of carazolol present in the urine of pigs and humans (lactate, acetate and glucuronide) are pharmacologically inert. All other available breakdown products were inactive, with the exception of the amine metabolite. However, it is weaker than carazolol by a factor of 10.

APPRAISAL

No additional residues studies were provided for cattle. A non-radiometric residue study in pigs was conducted to determine the depletion of carazolol in edible tissues over a 24-hour period. The residues at 2-hours post-administration were 56.8, 1.8, 6.9, 9.9, 1.8, and 2.5 µg/kg in the injection site, muscle, kidney, liver, fat and skin, respectively. Carazolol depleted rapidly in all tissues. Muscle and fat were at the limit of detection at 12 hours post-administration. All samples were at the limit of quantitation at 12 hours post-administration.

The radiometric study requested in pigs was not provided. Therefore, there is no additional information on the concentrations of carazolol and its metabolites as proportions of the total residues. A comparison of the results of this nonradiometric study with the previous radiometric studies in pigs provides limited information because only the 2-hour time point was in common in the design of the studies and only the proportion of parent carazolol to total residue can be determined. At the 2-hour depletion time, the majority of the total residue in muscle appears to be carazolol, however, 50-70% of the total residue is unidentified in fat, kidney, and liver.

The major metabolites of carazolol in the edible tissues of pigs have been demonstrated to not contribute significantly to the biological b-adrenoceptor-blocking activity of the residues. Based on the ADI of 0 - 0.1 µg/kg for parent carazolol established by the Committee, the permitted daily intake is 6 µg. MRLs can be established from the concentrations of carazolol at 2 hours post-administration as determined by the HPLC analyses: 1.8, 6.9, 9.9 and 1.8 µg/kg for muscle, kidney, liver and fat, respectively. MRLs are established such that essentially all the animals will have residue concentrations less than the MRLs when the drug is used according to the approved conditions of use. Using the HPLC values for carazolol at 2-hours post-administration and adding at least three statistical standard deviations, MRLs for parent carazolol in the tissue of swine are determined:

Muscle	5 µg/kg
Liver	25 µg/kg
Kidney	25 µg/kg
Fat/Skin	5 µg/kg

Using the above MRL values and the standard daily consumption of edible animal tissues of 300 g of muscle, 100 g of liver, and 50 g of each of kidney and fat, the theoretical daily intake of carazolol from these four

tissues would be 5.5 µg.

The Committee did not recommend MRLs for cattle.

REFERENCES

Delahaut, Ph. (1994). Residue study of carazolol after SUACRON™ 10 µg/kg single intramuscular dose in 16 pigs. Bio-Pharma reference: 193.551. Unpublished report submitted by Boehringer Mannheim, Praemix Wirkstoff GmbH, Sandhofer Strasse, 116, D-68305 Mannheim.

Rudolf, M. (1988). The beta-blocker carazolol and its metabolites - residue testing and metabolism. Dissertation for a Doctorate in Chemistry at Hamburg University.

DEXAMETHASONE

First draft prepared by
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RESIDUE DEPLETION STUDY

Background

During the 42nd Meeting of JECFA in Rome, 7th February 1994, the sponsors submitted fresh data to facilitate the establishment of MRLs in horses (Bette, 1994). These results suggest that residue depletion of dexamethasone-21-isonicotinate in horses following a single intramuscular administration of VOREN[®] suspension is in line with the findings previously presented for cattle and pigs.

Horses

Nine Welsh mountain ponies (1 control); age 14 years; ca. 300 kg, 1 male and 3 females per group were employed. VOREN[®] suspension (1 mg dexamethasone-21-isonicotinate/mL in an injectable suspension containing microcrystalline particles, 20 µg/kg body weight) was administered as a single intramuscular injection into the neck.

Samples of blood were taken 3 hours post dose and muscle, liver, kidney, fat, and injection site samples were acquired upon sacrifice. Dexamethasone in plasma and tissues were measured by HPLC/MS. Results are shown in Table 1.

Table 1 Residue Depletion of Dexamethasone-21-isonicotinate in Horses Following a Single Intramuscular Administration of VOREN[®] Suspension of 20 µg/kg BW

Days post dosing	Mean Concentration (µg/kg)*				
	Muscle	Liver	Kidney	Fat	Injection Site
3	b	b	0.57	b	1698.2 (4)
21	b	b	b	b	11.68 (3)

a Geometric mean, values below the Limit of Quantification (LOQ) have been included in the calculation as the LOQ, the resulting average residue concentrations reported as "< values" (figures in brackets denote the numbers of samples above the LOQ).

b All values are below the LOQ of the assay (0.5 µg/kg for muscle, kidney, fat; 2.5 µg/kg for liver).

c µg dexamethasone found per injection site (ca. 300 g).

Nine cross bred ponies (1 control); age 12 years; ca. 300 kg, 2 males and 2 females per group were employed. VOREN[®] depot (3 mg dexamethasone-21-isonicotinate/mL in an injectable suspension containing microcrystalline particles, 60 µg/kg body weight) was administered as a single intramuscular injection into the neck.

Samples of blood were taken 3 hours post dose and muscle, liver, kidney, fat, and injection site samples were acquired upon sacrifice. Dexamethasone in plasma and tissues were measured by HPLC/MS. Results are shown in Table 2.

Table 2. Residue Depletion of Dexamethasone-21-isonicotinate in Horses Following a Single Intramuscular Administration of VOREN[®] depot of 20 µg/kg BW

Days post dosing	Mean Concentration (µg/kg) ^a				
	Muscle	Liver	Kidney	Fat	Injection Site
3	<0.70 (3)	b	1.34	b	6722.0 (4)
28	b	b	b	b	51.9 (4)

a Geometric mean, values below the Limit of Quantitation (LOQ) have been included in the calculation as the LOQ, the resulting average residue concentrations reported as "<values" (figures in brackets denote the numbers of samples above the LOQ).

b All values are below the LOQ of the assay (0.5 µg/kg for muscle, kidney, fat; 2.5 µg/kg for liver).

c µg dexamethasone found per injection site (ca. 300 g).

Residue studies in horses with Dexafort were expected in the summer of 1994 (Bette, 1994) but have not been made available for this evaluation.

APPRAISAL

Based on the limits of quantification of the HPLC/MS method and on the kinetics of depletion of the marker residue from the remaining food commodities the following MRLs common to cattle, and swine were proposed at the 42nd JECFA meeting:

Commodity	MRL [µg/kg]	Marker Residue
Muscle	0.5	dexamethasone
Liver	2.5	dexamethasone
Kidney	0.5	dexamethasone
Milk (cattle)	0.3 µg/l	dexamethasone

The data supplied for horses are in line with the data for other previously studied species. Therefore it is recommended that the temporary MRLs for residues in horses be the same as those proposed at the 42nd JECFA meeting for cattle, and swine.

REFERENCES

Bette, P. (1994). Submission to the 42nd JECFA Meeting, Rome, 7th February 1994 in answer to a question to the sponsors by the Expert Committee.

Quirke, J.F. and Höstermann, D. Corrigendum to the above submission, June 12, 1995.

DIHYDROSTREPTOMYCIN STREPTOMYCIN

First draft prepared by
Dr. R.J. Heitzman
Compton, Newbury
Berkshire, United Kingdom

IDENTITY

Chemical names:

Streptomycin:

O-2-deoxy-(methylamino)- α -L-glucopyranosyl-(1 $\rightarrow$ 2)-O-5-deoxy-3C-formyl- α -L-lyxofuranosyl-(1 $\rightarrow$ 4)-N,N'-bis(aminoiminomethyl)-D-streptamine

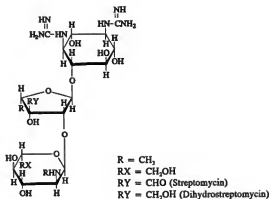
Dihydrostreptomycin:

O-2-deoxy-(methylamino)- α -L-glucopyranosyl-(1 $\rightarrow$ 2)-O-5-deoxy-3C-(hydroxymethyl)- α -L-lyxofuranosyl-(1 $\rightarrow$ 4)-N,N'-bis(aminoiminomethyl)-D-streptamine

Synonyms:

Many trade names

Structural formula:



Streptomycin:

Molecular formula:

$\text{C}_{21}\text{H}_{39}\text{N}_7\text{O}_{12}$ (Streptomycin A)

Molecular weight:

581.58

Molecular formula:

$\text{C}_{42}\text{H}_{68}\text{N}_{14}\text{O}_{24}\text{S}_3$ (Streptomycin sesquisulphate)

Molecular weight:

1457.4

Dihydrostreptomycin:

Molecular formula:

$\text{C}_{21}\text{H}_{41}\text{N}_7\text{O}_{12}$

Molecular weight:

583.62

Molecular formula:

$\text{C}_{42}\text{H}_{68}\text{N}_{14}\text{O}_{24}\text{S}_3$ (Dihydrostreptomycin sesquisulphate)

Molecular weight:

1461.4

OTHER INFORMATION ON IDENTITY AND PROPERTIES

Pure active ingredient:	Streptomycin or dihydrostreptomycin
Appearance:	White to grey or pale buff powder (streptomycin)
Melting point:	Dihydrostreptomycin sulphate, dec. at 250°C
Solubility:	Sesquisulphates are very soluble in water. Slightly soluble in alcohols.
Optical rotation:	$[\alpha]_D^{25} -88.5^\circ$ (1% soln of dihydrostreptomycin sulphate)
UV _{max} :	Streptomycin sulphate - 200 nm Dihydrostreptomycin sulphate - 202 nm

RESIDUES IN FOOD AND THEIR EVALUATION

CONDITIONS OF USE

Streptomycin and dihydrostreptomycin are active against a wide range of Gram-negative organisms and some Gram-positive pathogens in cattle, pigs and sheep. The combination of penicillin and streptomycin is especially useful in the treatment of mixed infections involving both Gram negative and Gram-positive organisms. Preparations of dihydrostreptomycin in combination with a penicillin derivative are used for the treatment and prevention of mastitis. The drugs may be prescribed for humans in certain second line treatments of infections.

Dosages

The preparations under consideration are administered as daily intramuscular injections (i.m.i.) at a dose rate of approximately 10 mg per kg body weight (BW) of streptomycin/Dihydrostreptomycin. They contain streptomycin or streptomycin plus dihydrostreptomycin or dihydrostreptomycin combined with a penicillin for horses, cattle, pigs or sheep. The length of treatment depends on the resolution of the infection and in the case of the procaine penicillin-G and dihydrostreptomycin preparations treatment should not exceed three to five days. There are licensed preparations containing dihydrostreptomycin usually combined with a penicillin which are administered into the mammary glands of cattle and sheep for the treatment or prevention of mastitis.

Preparations/Formulations submitted for evaluation

The sponsors (Norbrook) have submitted data for three preparations containing streptomycin or dihydrostreptomycin.

1. An aqueous solution of 200,000 units streptomycin sulphate per ml.
2. An aqueous solution of 120,000 units streptomycin sulphate and 120,000 units of dihydrostreptomycin sulphate per ml.
3. An aqueous suspension of 250 mg dihydrostreptomycin sulphate and 200 mg procaine penicillin per ml.

The sponsors (Pitman-Moore) have submitted data for their intramuscular injection preparation which is an aqueous suspension of 250 mg dihydrostreptomycin and 200 mg procaine penicillin-G per ml. They also license three preparations for intramammary infusion which contain both dihydrostreptomycin and procaine penicillin-G.

METABOLISM

Pharmacokinetics

Ten cattle (mean wt 467 ± 23 kg), six sheep (mean wt 44.8 ± 11.3 kg) and six pigs (mean wt 18.7 ± 6 kg) received daily for three days i.m.i. of streptomycin sulphate. The cattle and pigs received a dose of approximately 10,000 units streptomycin per kg BW and the sheep a dose of 8000 - 9500 units per kg BW. Blood samples were collected at frequent intervals after the first and third injection and assayed for streptomycin using a microbiological method (LOD 0.1 µg/ml). The results for plasma concentrations during the 24 hour period after the last (third) injection are shown in Figure 1 and the pharmacokinetic parameters are summarised

in Table 1. There is a more rapid uptake of streptomycin and also a shorter half-life of streptomycin in pigs than in cattle and sheep. In all three species the concentration of streptomycin is less than 1 µg/ml at 24 hours after the last (third) injection.

In a second study six calves (mean wt 136 ± 29 kg), six sheep (mean wt 39.2 ± 6.6 kg) and six pigs (mean wt 26.8 ± 1.8 kg) received daily for three days i.m.i. of streptomycin sulphate and dihydrostreptomycin sulphate. The animals received a dose of approximately 5,000 units streptomycin per kg and 5000 units dihydrostreptomycin per kg. Blood samples were collected at frequent intervals after the first and third injection and assayed for total streptomycin plus dihydrostreptomycin activity using a microbiological method (LOD 0.1 µg/ml). The results for plasma concentrations during the 24 hour period after the last (third) injection are shown in Figure 1 and the pharmacokinetic parameters are summarised in Table 1. In this study there were similar results for plasma levels and pharmacokinetic parameters for each of the species. The half-life activity of the combined preparation appears longer than for the streptomycin alone preparation. This is difficult to explain as being related to the dihydrostreptomycin component as the half life of dihydrostreptomycin in the combined preparation with procaine penicillin G is much shorter (see below).

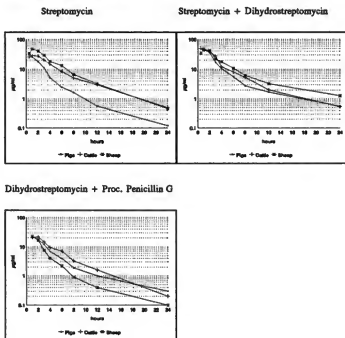
Table 1. Pharmacokinetic parameters of streptomycin in the plasma of farm animals after intramuscular injection of streptomycin sulphate or streptomycin sulphate plus dihydrostreptomycin sulphate.

Species i.m.i.	C _{max} (mg/l)	T _{max} (hours)	AUC (mg·h/l)	t _{1/2} (hours)
STREPTOMYCIN				
Cattle 1st	31 ± 9	1.4 ± 0.5	159 ± 26	2.5 ± 0.6
3rd	32 ± 10	1.2 ± 0.6	163 ± 28	3.1 ± 0.6
Sheep 1st	54 ± 6	1.0 ± 0	222 ± 21	2.6 ± 0.5
3rd	49 ± 5	1.2 ± 0.4	225 ± 23	2.9 ± 0.5
Pigs 1st	32 ± 4	0.75 ± 0.3	112 ± 18	1.9 ± 0.2
3rd	36 ± 10	0.5 ± 0	90 ± 17	1.8 ± 0.2
STREP+DIHYDROSTREP				
Calves 1st				
3rd	47 ± 15 56 ± 18	1.25 ± 0.6 1.08 ± 0.5	167 ± 17 157 ± 36	4.0 ± 0.7 3.7 ± 0.3
Sheep 1st	45 ± 12	0.83 ± 0.3	138 ± 25	4.4 ± 1.2
3rd	52 ± 13	1.08 ± 0.5	162 ± 33	3.8 ± 0.6
Pigs 1st	39 ± 10	1.25 ± 0.6	179 ± 63	4.0 ± 0.7
3rd	50 ± 15	1.22 ± 0.52	220 ± 68	4.4 ± 0.7
DIHYDROSTREP+ PROCAINE PENICILLIN G				
Cattle 1st	23.0 ± 5.7	1.4 ± 0.8	104 ± 21	2.7 ± 0.6
3rd	23.8 ± 3.2	1.5 ± 0.5	110 ± 28	2.6 ± 0.4
Sheep 1st	23.0 ± 6.4	1.7 ± 0.5	80 ± 18	1.6 ± 0.3
3rd	23.2 ± 5.0	1.0 ± 0	64 ± 16	1.8 ± 0.1
Pigs 1st	27.1 ± 9.3	1.2 ± 0.4	86 ± 13	2.3 ± 0.2
3rd	23.2 ± 6.1	1.3 ± 0.5	87 ± 18	2.3 ± 0.3

A comparison of levels of streptomycin in plasma and tissue cage fluid (TCF) was carried out in five calves (mean wt 147 ± 12 kg) implanted with polythene practice golf balls. The calves were administered by i.m.i. 10,000 units streptomycin sulphate per kg BW daily for three days. Plasma and TCF was collected at 6, 12 and 24 hours after each injection and assayed for streptomycin by a microbiological method. The concentration of streptomycin in the TCF was between 1 - 2.8 times higher than in the peripheral plasma which may indicate

a high degree of penetration of streptomycin into tissue.

Figure 1. Plasma concentrations of streptomycin/dihydrostreptomycin in farm animals after a third daily intramuscular injection. Time 0 is at the time of the third injection and 48 hours after the first daily injection.



Metabolism in Food Animals

No data was provided by the sponsors on the metabolism of streptomycin.

Metabolism in Toxicological Test Species

No data was provided by the sponsors on the metabolism of streptomycin.

In a review of comparative metabolism in food-producing animals Juskevitch (1987) stated that "It appears that there is minimal metabolism of the tetracyclines and aminoglycosides in ruminant species."

TISSUE RESIDUE DEPLETION STUDIES

Radiolabeled Residue Depletion Studies

Streptomycin

No data was provided by the sponsors for the radiolabeled depletion of the residues of streptomycin.

Dihydrostreptomycin

Dihydrostreptomycin, radiolabeled uniformly with tritium, was administered to cattle and pigs by i.m.i. at 25 mg/kg BW (Stalheim, 1970). The work did not identify the metabolites or the extent of metabolism but concentrated on the determination of the total activity and biological activity in blood and urine. The work showed that there was an exponential decrease in the concentration of radioactivity and antibacterial activity in plasma of both species. Most of the activity was excreted at an exponential rate into the urine within 12 hours and was 72% of dose in the case of the pigs. Nevertheless residues were still detected in porcine urine at 86 hours (3.1 mg/l) and in bovine urine at 96 hours (1.7 mg/l). There was good agreement between the measurements for the concentrations of total radioactivity and the antibacterial activity measured by bioassay. The LOD for the bioassay was 0.4 mg/l and was estimated as 8 µg/l for the radioassay. No activity was detected in bovine saliva or lacrimal fluid. The values of apparent volume of distribution (21% and 23%) agree with estimates for extracellular water. These results together with the lack of activity in both saliva and lacrimal fluids indicate that dihydrostreptomycin neither equilibrates with intracellular fluids nor accumulates intracellularly.

Other residue depletion studies (with unlabeled drug)

A series of residue studies in the edible tissues were carried out as outlined in Table 2.

Table 2. Studies for the determination of residues in edible tissues.

Species	Mean BW $\pm$ SD (kg)	n per time point	Dose i.m.l. & no. days† (units/kg BW or mg/kg BW)	Tissue	Sampling times after last injection (days)	LOD (ng/kg mg/l)
STREP						
Cattle	157 $\pm$ 52 181 $\pm$ 64	4 4	10,000 [3] 25,000 [1]	edible edible	7, 14 14, 21	1 1
Sheep	40 $\pm$ 2.6	4	10,000 [3]	edible	7, 14	1
Pigs	24.9 $\pm$ 4.3	4	10,000 [3]	edible	7, 14	1
STREP +	DIHYDROSTREP					
Calves	130 $\pm$ 27 156 $\pm$ 33	4 4	10,000 [3] 25,000 [1]	edible edible	7, 14, 21 21, 28	1 1
Sheep	42 $\pm$ 10	4	10,000 [3]	edible	7, 14, 21	1
Pigs	24 $\pm$ 4	4	10,000 [3]	edible	7, 14, 21	1
DISTREP +	PROC. PEN G					
Cattle	184 $\pm$ 81 100 $\pm$ 13	4/3 4	10,000 [3] 10,000 [3]	edible edible	7, 18 28	1 0.1
Calves	100 - 165	3/4	10 mg [5]	M,IS,K	6, 10, 14	0.05
Calves ^p	180 - 230	4	10 mg [5]	M,IS,K	21, 28	0.05
Cows ^M		2-5	10 mg [1]	edible	2h - 68h	0.3
Sheep	51 $\pm$ 17	4/3	10,000 [3]	edible	7, 18	1
Sheep	29 $\pm$ 2	4	10,000 [3]	edible	28	0.1
Sheep ^p	27 - 112	3/4	10 mg [5]	M,IS,K	5, 7, 10, 14	0.05
Sheep ^p	34 - 84	4	10 mg [5]	M,IS,K	21 - 28	0.05
Pigs	50 $\pm$ 38	4/3	10,000 [3]	edible	7, 18	1
Pigs	44 $\pm$ 2	4	10,000 [3]	edible	28	0.1
Pigs ^p	23 - 27	3/4	10 mg [5]	M,IS,K	6, 10, 14	0.05
Pigs ^p	19 - 155	4	10 mg [5]	M,IS,K	21, 28	0.05

^p are studies by Pitman-Moore, ^M uses a Mycopharm preparation (Nouws & Ziv, 1977), the others are by Norbrook; M = muscle, IS = injection site muscle and K = kidney

Edible tissues were heart, liver, muscle, injection site, kidney, kidney fat and subcutaneous fat. The residues were assayed by microbiological methods or in the Pitman-Moore studies, EIA was used.

In the Norbrook studies residues were detected in the edible tissue samples especially for the liver and kidney tissues collected at 7 days (and 14 days for the combined streptomycin and dihydrostreptomycin preparation) after the last injection. The residues in liver and kidney are shown in Table 3.

Table 3. Residues of antimicrobial activity (streptomycin - Strep and dihydrostreptomycin - DiStrep) (Mean values for 4 animals in mg/kg) in liver and kidney sampled at 7 and 14 days after the last injection (see Table 2 for doses).

Tissue	Withdrawal time (days)	Cattle	Sheep	Pigs
Liver				
Streptomycin	7	1.17	1.04	1.38
Strep + DiStrep	7	1.2	2.2	3.4
Strep + DiStrep	14	1.1	1.4	<1
DiStrep + Proc.Pen G	7	2.25	8.38	3.87
Kidney				
Streptomycin	7	1.07	1.12	1.84
Strep + DiStrep	7	4.6	13.0	16.5
Strep + DiStrep	14	3.5	2.5	5.9
DiStrep + Proc.Pen G	7	10.5	15.8	12.6

Streptomycin preparation:

There were NO residues in tissues other than liver and kidney at 7 days post injection and there were NO residues detected in any edible tissue at time points 14 days or later (see Table 2 for times).

Streptomycin/Dihydrostreptomycin preparation:

In cattle there were NO residues in tissues other than liver and kidney at 7 and 14 days post injection. In sheep there were NO residues in tissues other than liver and kidney at 7 and 14 days post injection except residues were detected at the injection site at 7 days in one sheep (2.8 mg/kg) and one sheep (2.8 mg/kg) at 14 days. One sheep muscle sample taken at 7 days was also positive (1.3 mg/kg).

In pigs there were NO residues in tissues other than liver and kidney at 7 and 14 days post injection except residues were detected at the injection site at 14 days in one pig (1.4 mg/kg). One porcine subcutaneous fat sample taken at 7 days was positive (3.3 mg/kg) and one porcine kidney fat sample was positive (1.4 mg/kg) at 14 days.

Dihydrostreptomycin/Procaine penicillin G preparation:

Residues were present in most tissues at 7 days after three daily injections (see Table 4). The next sampling point was 18 days post injection and NO residues were detected in any edible tissue at this time (Norbrook studies).

In the Pitman-Moore studies residues of dihydrostreptomycin in cattle, sheep and pigs were measured only in kidney, muscle and injection site muscle. Residues were present in the muscle at day 7 and were <0.07 mg/kg at day 14. Residues were found in kidney and at injection site on days 21 and 28 although they were <1 mg/kg on day 28. The results are shown in Table 5.

Table 4. Samples with positive ($\oplus$) and no (-) microbiological activity (LOD ≥ 1 mg/kg) for dihydrostreptomycin 7 days after last injection of dihydrostreptomycin / procaine penicillin G (see Table 2 for doses).

Tissue	Cattle	Sheep	Pigs
Muscle	----	$\oplus$ ---	$\oplus$ ---
Inj. site	$\oplus$ ---	$\oplus \oplus \oplus \oplus$	----
Heart	----	$\oplus \oplus \oplus \oplus$	$\oplus \oplus$ --
Liver	$\oplus \oplus \oplus \oplus$	$\oplus \oplus$ --	$\oplus \oplus$ --
Kidney	$\oplus \oplus \oplus \oplus$	$\oplus \oplus \oplus \oplus$	$\oplus \oplus \oplus \oplus$
Kidney fat	----	$\oplus \oplus \oplus$	$\oplus$ ---
Subcut. fat	----	$\oplus \oplus \oplus$	$\oplus$ ---

* Only three samples.

Table 5. Residues (mg/kg) of dihydrostreptomycin in tissues of farm animals after 5 daily intramuscular injections of dihydrostreptomycin combined with procaine penicillin-G (see Table 2 for doses).

Species	No.	Days after dosing	Muscle	Injection Site	Kidney
Cattle	4	6	0.28 - 1.25	0.56 - 3.52	10.8 - 56.3
	2	10	<0.05, 0.65	0.07, 2.08	7.04, 12.80
	4	14	<0.05 - 0.05	0.80 - 1.60	5.12 - 19.2
	4	21	n.m.	<0.88 - 2.28	1.75 - 1.96
	4	28	n.m.	<0.28 - <0.8	<0.40 - 0.46
Sheep	4	5	0.07 - 0.18	1.52 - 19.2	6.72 - 16.6
	2	7	0.12, 0.13	1.04, 2.08	4.00, 4.16
	4	10	0.08 - 0.10	0.30 - 2.24	3.04 - 5.44
	4	14	<0.05 - 0.07	0.88 - 2.40	1.00 - 1.52
	4	21	n.m.	1.16 - 5.64	0.05 - 0.93
	4	28	n.m.	<0.1 - <0.2	<0.3 - <0.8
Pigs		6	0.06 - 2.32	0.10 - 1.28	8.96 - 26.2
		10	0.05, 15.7	0.84, 1.04	3.84, 4.16
		14	<0.5 - 0.5	0.21 - 0.56	1.40 - 4.32
		21	n.m.	<0.8 - 0.94	0.96 - 1.92
		28	n.m.	<0.1 - <0.4	<0.1 - <0.4

Data from Pitman-Moore; n.m. not measured

Nouws and Ziv (1977) reported residues of dihydrostreptomycin in kidney and urine 68 hours after i.m. injection of dihydrostreptomycin/Procaine penicillin-G but NO residues were detected (LOD 0.2 - 0.6 mg/kg) in muscle, liver, serum or bile.

Milk

Residues of dihydrostreptomycin and streptomycin in the milk of cows treated with a variety of intramuscular and intramammary preparations containing dihydrostreptomycin were measured by EIA (Pitman-Moore (AnH89/R/87); Dötsch, 1993; Usleber et al., 1993, 1994) or bioassay (Norbrook file; Moretain and Boisseau, (1993). The times to reach levels below the MRL (200 µg/l, 12th JECFA) and the limits of detection for each study are shown in table 6. The persistence of the residues depends on the formulation of the preparation.

Table 6. Depletion of dihydrostreptomycin and streptomycin in milk of treated cows

Route Study	Dose mg/kg BW * g total	No. cows	Milkings MRL Mean	to reach 200 µg/l Max	Milkings LOD Mean	to reach Max	LOD (µg/l)
Intramuscular Pitman-Moore	10 ^D x 5	10	1	3	> 14	> 14	5
Dötsch	12.5 ^D	9	5	6	12	21	19
Dötsch	12.5 ^{ODC}	12	7	8	13	19	19
Norbrook	10 ^S x 3	10	3 - 4	> 4	3 - 4	> 4	200
Norbrook	25 ^S	5	3	3	4	4	100
Norbrook	10 ^M x 3	5	2	3	4	4	100
Norbrook	25 ^M	5	3	4	4	4	100
Norbrook	10 ^D x 3	6	2	3	3	4	100
Intramammary Moretain ^S	0.35 g ^{OD}	5	14	15	14	15	150
Moretain ^S	0.1 g ^{OD}	5	5	7	8	11	150
Usleber ^D	0.5 g ^{ODC}	6	5	6	16	24	19
Usleber ^D	2 g ^{ODC}	1	13	-	> 26	-	19
Usleber ^D	0.5 g ^{OD}	6	5	6	16	24	19

^D data from Moretain & Boisseau (1993), ^S data from Usleber et al., (1994) and Dötsch (1993).

DS is dihydrostreptomycin; ^S is streptomycin; ^M is DS combined with streptomycin; ^D is DS combined with penicillin G; ^{ODC} is DS combined with penicillin G, dexamethasone and chlorpheniramin; ^{OD} is DS combined with penicillin G, kanamycin, disphenylsulphone and prednisolone.

LOD is Limit of Detection.

Poultry

Nakamura et al (1967) studied the distribution of dihydrostreptomycin (in combination with potassium penicillin-G) in chickens for periods ranging from 10 min up to 24 h following oral dosing. They assayed the residues by bioassay and showed that the maximum levels were recorded during the first two hours after administration and that the highest concentrations were in the bile with the levels in the tissues in the following decreasing order; pancreas > kidney > serum > spleen > muscle. No residues were found in the brain, heart or testes.

Eggs

The depletion of dihydrostreptomycin in eggs was reported and showed that after a single i.m. injection of 100 mg/kg BW, residues were above 500 µg/kg in whole egg for at least 8 days after injection (Roudaut, 1989). Laying hens were also administered the drug in the drinking water at level of 1 g/l, equivalent to 100 mg/kg BW, for five consecutive days. No antimicrobial residues in eggs were detected (LOD 300 µg/kg albumin, 3000 µg/kg yolk) suggesting a very low absorption of the drug from the gastrointestinal tract.

Bound Residues and Bioavailability

No data was submitted by the sponsors.

METHODS OF ANALYSIS FOR RESIDUES IN TISSUES

Overview

Several types of analytical methods are used for the assay of the aminoglycosides, especially in the analysis of body fluids. Immunochemical and microbiological assays are the best suited for rapid screening and work most successfully with milk samples. Chromatographic methods using either thin layer chromatography (TLC) or high performance liquid chromatography (HPLC) are suitable for the analysis of streptomycin and dihydrostreptomycin but have not been adequately developed for the analysis of residues in edible tissues.

The sponsors have used specific microbiological assays for measuring both streptomycin and dihydrostreptomycin. The methods do not distinguish between the two compounds. The older method from Norbrook laboratories (1988 et seq) uses the organism *Bacillus subtilis* NCIB 8054 and has a limit of detection of 1 mg/kg. A newer method uses *Bacillus subtilis* ATCC 6633 with a limit of detection of 0.1 mg/kg. There is no interference from penicillins. The methods are partially validated for the measurement of recovery from spiked samples of muscle, liver and kidney for both methods. Although results using both methods are given for residues in heart, injection site, subcutaneous fat, kidney fat and skin only the newer method was validated for fat and skin. The recoveries using both methods vary with both the type of tissue and the mass of spike. Although not given the limit of quantification must be considerably greater than the limit of detection. No repeatability data is provided for incurred samples. The results are not compared with other non-microbiological assays.

Outline of Methods

Assay using *Bacillus subtilis* NCIB 8054 and streptomycin, (Sponsor). Five grams of tissue are homogenised in phosphate buffer, pH 1.5. Spikes of streptomycin equivalent to 1-5 mg/kg tissue were added at this point for the validation of the assay in muscle, liver and kidney. After an equilibration period of 30-45 min the mixture is adjusted to pH 8.0 with 20% sodium hydroxide and equilibrated for a further 30 min. The mixture is centrifuged to remove the tissue debris and denatured proteins and the supernatant is collected and used for the microbiological assay. The assay uses *Bacillus subtilis* NCIB 8054 as the test medium and incorporates penicillinase to destroy any penicillins present in the extract. A calibration curve was prepared using a pure standard of streptomycin dissolved in buffer. The concentration in the test sample was calculated by interpolation with the standard curve and using a recovery factor of 60% for all tissues. The percentage recoveries $\pm$ SD at the 1 mg/kg level were, muscle 56 ± 18 , liver 65 ± 10 and kidney 54 ± 13 for at least 10 replicates per tissue.

Assay using *Bacillus subtilis* ATCC 6633 and dihydrostreptomycin, (Sponsor). Ten grams of tissue are homogenised in phosphate buffer, pH 2.0. Spikes of dihydrostreptomycin equivalent to 0.1, 0.2 and 0.5 mg/kg tissue were added after centrifugation for the validation of the assay in muscle, liver, kidney, fat and skin. After an equilibration period of about 45 min. the mixture is adjusted to pH 8.0 with ethanolamine and equilibrated for a further 30 min. The mixture is centrifuged to remove the tissue debris and denatured proteins and the supernatant is collected and used for the microbiological assay. The assay uses *Bacillus subtilis* ATCC 6633 as the test medium and incorporates penicillinase to destroy any penicillins present in the extract. A calibration curve was prepared using a pure standard of dihydrostreptomycin dissolved in buffer. The concentration in the test sample was calculated by interpolation with the standard curve and using recovery factors of 78, 75, 82, 85 and 76% respectively for muscle, liver, kidney, fat and skin tissues. These recovery factors are the mean value for the recoveries of the three different levels of spikes. The percentage recoveries $\pm$ SD at the 0.1 mg/kg level were, muscle 70 ± 12 , liver 70 ± 9 , kidney 69 ± 1 , fat 73 ± 3 and skin 79 ± 2 for an unstated number of replicates per tissue.

Assay using *Bacillus pumilis* and dihydrostreptomycin, (Pitman-Moore). Tissues are incubated for 30 min at 37°C with phosphate buffer, pH 8.0 containing lipase. The sample is centrifuged and the supernatant used for bioassay. Spikes of dihydrostreptomycin were added for the validation of the assay in muscle, injection site muscle, kidney and milk. The LOD in muscle, liver and fat is 0.31 mg/kg, in kidney 0.62 mg/kg and for milk it is 5 µg/l.

A calibration curve was prepared using a pure standard of dihydrostreptomycin dissolved in buffer. The concentration in the test sample was calculated by interpolation with the standard curve and using recovery factors of 78, 75, 82, 85 and 76% respectively for muscle, liver, kidney, fat and skin tissues. These recovery factors are the mean value for the recoveries of the three different levels of spikes. The percentage recoveries $\pm$ SD at the 0.1 mg/kg level were, muscle 70 ± 12 , liver 70 ± 9 , kidney 69 ± 1 , fat 73 ± 3 and skin 79 ± 2 for an unstated number of replicates per tissue.

Immunochemical Method for Residues in Milk using Enzyme Immunoassay (EIA)

Pitman-Moore method

1 gram of tissue was homogenised in phosphate buffered saline (PBS), pH 7.0. After centrifugation an aliquot of the supernatant was used for an EIA using polyclonal antibodies specific for streptomycin and dihydrostreptomycin. There was no cross-reaction with other antibacterial drugs including other aminoglycosides. Because of matrix effects standard curves were prepared by spiking tissues prior to extraction. No measurements of residues were made for liver, fat or skin. Milk was assayed after dilution with PBS. The LOD for milk was 5 $\mu\text{g/l}$ and varied for tissues from 140-800 $\mu\text{g/kg}$. The method was not done to GLP or accredited.

In a method from Germany (Usleber et al, 1994) defatted milk was diluted in phosphate buffered saline and directly assayed by EIA using rabbit antibodies and horse-radish peroxidase conjugates. The measuring range of the standard curves was 0.2 to 50 $\mu\text{g/l}$ with 50% inhibition about 5 $\mu\text{g/l}$, CVs were <7.5% and the LOD was 20 $\mu\text{g/l}$. The method is suitable for monitoring the current MRL of 200 $\mu\text{g/l}$.

APPRAISAL

The aminoglycosides streptomycin and dihydrostreptomycin were evaluated together because their pharmacokinetic and residue patterns were similar and also they were not distinguished from each other in the measurement of residues by either microbial inhibition assays or enzyme immuno assay (EIA) methods.

Both drugs were absorbed rapidly from the site of intramuscular injection in cattle, sheep and pigs and were thought to be distributed in the extracellular fluids. They were cleared rapidly from the blood.

There was very little information on the metabolism in farm animals of either compound although probably the drugs are not substantially metabolised.

There were no radiodepletion studies reported to determine the total residues in the tissues.

Numerous residues studies using unlabeled drug(s) were presented in a variety of preparations. The residues in these studies were measured in cattle, sheep and pigs following intramuscular injections at the recommended doses. The residues were measured by either microbial inhibition assays or by EIA methods which do not distinguish between the two compounds and may indeed cross-react with possible metabolites. The highest and most persistent residues were found in the kidney. Residue levels in muscle, liver and fat depleted fairly rapidly to below the level of detection (0.03 to 1 mg/kg varying with tissue and study). There was wide variability in the disappearance of the residues depending mainly on the formulation of the preparation. Dihydrostreptomycin was retained in the injection sites of cattle, sheep and pigs in low amounts (<0.8 - 5.6 mg/kg) for three weeks but was <0.8 mg/kg at four weeks. Residues of dihydrostreptomycin and streptomycin in the milk of cows treated with a variety of intramuscular and intramammary preparations containing dihydrostreptomycin were measured by EIA, a receptor binding assay or microbial inhibition assays. The times to reach levels below the MRL (200 $\mu\text{g/kg}$, 12th JECFA) for each study varied between 3 and 15 milkings after drug administration. Again the persistence of the residues depends on the formulation of the preparation. Kidney, muscle and milk are recommended as target tissues. The depletion of dihydrostreptomycin in eggs was reported and showed that after a single i.m. injection of 100 mg/kg BW residues were above 500 $\mu\text{g/kg}$ in whole egg for at least 8 days after injection. Laying hens were also administered the drug in the drinking water at level of 1 g/l, equivalent to 100 mg/kg BW, for five consecutive days. No antimicrobial residues in eggs were detected (LOD 300 $\mu\text{g/kg}$ albumin, 3000 $\mu\text{g/kg}$ yolk) suggesting a very low absorption of the drug from the gastrointestinal tract.

No data were presented on bioavailability of bound residues.

The analytical microbial inhibition assays and EIA methods for measuring residues are adequate for screening purposes at sensitivities well below 1 mg/kg for tissues. In milk the EIA methods have claimed limits of detection (LOD) of 5-20 µg/l whereas in the microbial inhibition assays the LOD are 100-200 µg/l. Although much data was presented neither method was fully validated nor in the case of the EIA done to Good Laboratory Practice (GLP) standards. Chemical methods for measuring aminoglycosides based on chromatography were reported in the literature for assaying residues in animal tissues. LOQs were 20 µg/kg for streptomycin and 40 µg/kg for dihydrostreptomycin for muscle and kidney (Gerhardt et al., 1994a) and half of these values for milk (Gerhardt et al., 1994b). An LC/MS/MS method was reported for measuring residues in bovine kidney with LOQs of 440 µg/kg and 320 µg/kg for streptomycin and dihydrostreptomycin, respectively.

Maximum Residue Limits

Based on the temporary ADI of 0 - 30 µg/kg for the parent drugs established by the Committee, the permitted daily intake of the parent drugs and/or their equivalents is 1800 µg for a 60 kg person per day.

The following factors were considered in estimating the MRLs:

1. The temporary ADI which is established on a toxicological basis.
2. The limits of detection or quantitation of the methods.
3. The marker residue is the sum of streptomycin and dihydrostreptomycin for edible tissues and milk.
4. Insufficient data was presented for residues in eggs to maintain the MRL set at the 12th JECFA meeting.
5. There is an uncertain ratio of the marker residues to the total residues although it is predicted that there is little metabolism of either drug and the ratio may be close to unity.
6. MRLs established following the 12th JECFA meeting were; meat 1000 µg/kg, milk 200 µg/kg and eggs 500 µg/kg.

The Committee recommends temporary MRLs of 500 µg/kg for muscle, liver and fat and 1000 µg/kg for kidney of cattle, sheep, pigs and chickens and 200 µg/l for cattle milk expressed as the sum of streptomycin and dihydrostreptomycin. Using these values for the MRLs and taking into account the factors above, the ingested residue of parent drug are muscle (300 g) 150 µg, liver (100 g) 50 µg, kidney (50 g) 50 µg, fat (50 g) 25 µg and milk (1.5 liters) 300 µg. The total is 575 µg/day.

The Committee requests further information, either experimentally or as an expert report, on the metabolism of the drugs and the total residues in farm animals. Additional residue data is required for eggs. The relationship between the antimicrobial activity of the residues and the measurement of residues by specific chemical methods is requested.

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ENROFLOXACIN

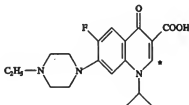
First draft prepared by
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Compton, Newbury
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IDENTITY

Chemical name: 1-cyclopropyl-7-(4-ethyl-1-piperazinyl)-6-fluoro-1,4-dihydro-4-oxo-3-quinoline carboxylic acid (hydrochloride)

Synonym: BAY Vp 2674

Structural formula:



[Note: Ciprofloxacin is formed by the loss of the ethyl group in the piperazine ring;
* C-2 position of ^{14}C radiolabel]

Molecular formula: $\text{C}_{19}\text{H}_{22}\text{FN}_3\text{O}_3$

Molecular weight: 359.4

OTHER INFORMATION ON IDENTITY AND PROPERTIES

Pure active ingredient:	Assay min. 98%
Appearance:	Pale yellowish to light yellow crystalline substance
Melting point:	decomposes
Solubility (g/100 ml):	Water pH 7 0.01; ethanol 0.20; 0.1M HCl 0.17; 0.1M NaOH 1.19
UV_{max}:	277 nm in 0.1M HCl
Stability:	3 years

RESIDUES IN FOOD AND THEIR EVALUATION

CONDITIONS OF USE

Enrofloxacin is a drug with a wide spectrum of activity against bacteria. The major metabolite formed in farm species is ciprofloxacin, an antibacterial drug used in human medicine.

Dosages

Enrofloxacin may be administered orally or parentally to calves and pigs at a dose of 5 mg/kg bodyweight (BW) and orally to poultry at a dose of 10-12 mg/kg BW. There is some use in lactating dairy cows and laying hens. The normal treatment period is 3 to 5 days.

METABOLISM

The radiolabeled compound used for the pharmacokinetic, metabolism and residue depletion studies was 2-¹⁴C-enrofloxacin except in two studies, one in rats (Bayer report PF 3784) and another in broiler chickens (Bayer Report PF 3963) in which piperazine-2,3-¹⁴C-enrofloxacin was used. The radiochemical purity of the batches was between 96.4 % and 99.2 %

Pharmacokinetics

Calves

Radiolabeled enrofloxacin was administered as a single oral dose of 5 mg/kg BW. The concentrations of radiolabel in blood cells and plasma reached peak values of about 1 mg drug equivalents per l or kg eight hours after administration. Biological activity (bioassay method) in blood peaked at 6-8 h (Bayer Report 73208).

Poultry

After administration of 2-¹⁴C-enrofloxacin in multiple oral doses of 12 mg/kg body weight daily for 7 days to chickens peak blood levels of radioactivity were reached in less than 6 h (Bayer Report 73185 Table 4). The elimination half-life in plasma was about 1.4 days.

Piperazine-2,3-¹⁴C-enrofloxacin (SA 2.21 MBq/mg) was administered orally to broiler chickens at a dose level of 5 mg/kg body weight on seven consecutive days (Bayer Report PF 3784). 87.4 % of the totally administered dose was excreted within 150 hours of the first administration, i.e. 6 hours after last administration. The excretion during the 24 hour period after the last dosage was 12-15 % of the final dose.

Rats

Enrofloxacin was rapidly absorbed following oral administration of 2-¹⁴C-enrofloxacin to rats and about 75 % of the compound was bioavailable. The peak concentration in blood was reached in under 30 minutes with an elimination half-life of 11.7 ± 2.45 h. After intravenous (i.v.) injection of enrofloxacin the elimination half-life was 7.91 ± 0.65 h. There was a rapid elimination of the radiolabel into the bile reaching a plateau value of 40 % of the oral dose by 24 h (Bayer Report 73217).

Piperazine-2,3-¹⁴C-enrofloxacin (SA 2.21 MBq/mg) was administered orally to 15 male Wistar rats at a dose level of 5 mg/kg body weight on seven consecutive days (Bayer Report PF 3784). The excretion was fast and mainly faecal (ca. 68 % of the dose within 24 hours after the first dosage).

Metabolism

Rats

Rats (Bayer Reports 73217, 73349 and PF 3784) and farm animals (73296, 73288, 73413, 73380 and PF 3963) were administered orally ¹⁴C-enrofloxacin. The results are summarised in Table 1. In all the samples parent drug and ciprofloxacin were the major residues except in poultry muscle and skin in which only parent drug but not ciprofloxacin was present. This is in contrast to the metabolism in the bovine where ciprofloxacin is the most abundant residue. In rat urine enrofloxacin glucuronide was a major metabolite. In the edible tissues several other metabolites were detected. These minor metabolites were not identified and usually each

metabolite represented <2% of the total residue. The non-extractable residues formed a significant proportion of the residues in muscle, liver, kidney, fat and poultry skin. The antimicrobial activity of the residues other than parent drug and ciprofloxacin were not determined.

Metabolism in Food Animals

Cattle

Two calves aged 2-4 weeks, were administered by oral gavage ^{14}C -enrofloxacin at a rate of 5 mg/kg BW for seven consecutive days. The calves were sacrificed at twelve hours and 72 hours after the final dose and muscle, liver, kidney and fat samples were examined for the characterisation of the radiolabeled residues (Bayer Report, 73296). Enrofloxacin and ciprofloxacin were the major residues. Unlike the other species examined in which enrofloxacin is the major residue, in these calves ciprofloxacin was present in similar amounts to the parent drug. The results are shown in Table 2. Similar results were obtained in the residue depletion studies using unlabeled drug (see Table 7.)

Pigs

One pig, body weight 20-25 kg, was administered by oral gavage ^{14}C -enrofloxacin at a rate of 5 mg/kg BW for seven consecutive days. Twelve hours after the final dose the pig was sacrificed and muscle, liver, kidney and fat were examined for the characterisation of the radiolabeled residues using either HPLC or TLC separation. There was a good agreement between the chromatography methods and enrofloxacin and ciprofloxacin accounted for more than 80% of the total residues in the four tissues (see Table 2). No other metabolites were identified (Bayer Report, 73288).

Table 1. Major metabolites of enrofloxacin found in farm animals.

Metabolite		Rat	Cow	Pig	Poultry
Parent (Bay Vp 2674)	Urine	+++			
	Bile	+++			
	Liver	+++	++	+++	+++
Ciprofloxacin (Bay O 9867)	Urine	+++			
	Bile	+			
	Liver	+	+++	+	++
M2 (Parent glucuronide ?)	Urine	++			
	Bile	++			
	Liver		?		?
M6	Urine	+			
	Bile				
	Liver				
Bay p 9357	Urine				
	Bile				
	Liver				++
Non-extractable	Liver	n.m.	++	+	++ (6h) ++ ^C (3-10d) +++ ^T (3d) 0 ^{C2} (6h)

Total residues; - +++ >25%, ++ 10-25%, + 2-10%, n.m. is not measured
Result for ^C chickens (Bayer report 73185), ^{C2} chickens (Bayer report PF 3963), ^T turkeys,
other poultry results are for both species.

Poultry

The study on chickens was done with 30 day-olds and the study for turkeys with 21 day-olds. For each species

three birds per group in four groups of birds were administered orally 2-¹⁴C-enrofloxacin at a rate of 12 mg/kg BW for 3, 5, 7 and 10 consecutive days, respectively. The birds were sacrificed at six hours after the final dose. The samples for birds dosed 3 and 5 days were combined as also were the samples for 7 and 10 days dosing and samples of muscle, liver and skin with adhering fat were examined for the characterisation of the radiolabeled residues. In the first two studies (Bayer Reports, 73179 and 73292) enrofloxacin and ciprofloxacin were the major residues in liver but only parent drug was present in muscle and skin. However there was incomplete extraction of the residues and the studies were repeated in which >90% of the residues were extracted (Bayer Reports, 73413 and 73380).

The results from the repeated studies for the combined 7 and 10 days dosed birds are shown in Table 2. In all tissues enrofloxacin was the major residue and ciprofloxacin was the major metabolite. Most of the remaining residue not accounted for was either insoluble in the HPLC mobile phase or not detected in the HPLC due to interfering lipophilic substances.

Piperazine-2,3-¹⁴C-enrofloxacin (SA 2.21 MBq/mg) was administered orally to broiler chickens at a dose level of 5 mg/kg body weight on seven consecutive days (Bayer Report PF 3784). The birds were sacrificed 6 hours after the last dose and metabolism was investigated in excreta and body tissues. The concentrations of residues were 1.24 mg/kg in liver, 0.50 mg/kg in kidney, 0.25 - 0.30 mg/kg in muscles and 0.13 - 0.15 mg/kg in fat. The radioactivity in the edible tissues was readily extractable with acetonitrile and water with recoveries ranging from 86 - 104%. In liver, fat, kidney and muscle enrofloxacin accounted for more than half the total residues. Ciprofloxacin was a major residue in liver (24%) and kidney (24%) and a minor residue in muscle and fat (3 - 6%). The presence (11%) of a unique metabolite, the N-hydroxy derivative formed after N-dealkylation was found in the liver. Another metabolite (5%) was found in fat and was formed by the opening of the piperazine ring.

Table 2. Characterisation of residues of ¹⁴C-enrofloxacin in farm species.

Tissue Species	WT (days)	Total equivalents(µg /kg)	ENX (% total)	CIPX (% total)	Non-extractable (% total)
Liver					
Calf	0.5	9170	24	39	12
Calf	3.0	3860	c.7	14	17
Pig	0.5	2975	73	8.3	-
Chicken	0.25	2060	66	13	2
Turkey	0.25	5220	40	5	7
Kidney					
Calf	0.5	4870	33	41	1
Calf	3.0	370	?	27	11
Pig	0.5	2943	82	5.7	-
Muscle					
Calf	0.5	3680	48	41	2
Calf	3.0	120	17	42	33
Pig	0.5	1564	81	3.4	-
Chicken	0.25	2610	79	3	9
Turkey	0.25	1600	54	0	3
Fat					
Calf	0.5	3050	47	35	(?)
Calf	3.0	300	43	20	17
Pig	0.5	293	75	3.6	-
Skin					
Chicken*	0.25	1330	50	4	4
Turkey*	0.25	1000	70	0	9

* See also Table 4 for more values; ENX is enrofloxacin; CIPX is ciprofloxacin

Metabolism in Toxicological Test Species

Rats were administered a single oral dose of 2-¹⁴C-enrofloxacin of 5 mg/kg BW (Bayer report 73217) or daily oral doses of 165 mg/kg BW for 3 days (Bayer reports, 73349, 73379 and 73474). Urine and bile were examined and the major residues were parent drug, ciprofloxacin and enrofloxacin glucuronide and M6 (6%) an unidentified metabolite. Other minor metabolites, (each <2%) were not identified.

Piperazine-2,3-¹⁴C-enrofloxacin (SA 2.21 MBq/mg) was administered orally to 15 male Wistar rats at a dose level of 5 mg/kg body weight on seven consecutive days (Bayer Report PF 3784). The rats were sacrificed 6 hours after the last dose. Metabolism was investigated in faeces, urine and body tissues. Enrofloxacin and ciprofloxacin were identified as the major metabolites as shown in Table 3. The highest concentrations of residues were observed in the liver (1.2 mg/kg) with lower amounts in kidney (0.68 mg/kg) and muscle (0.24 mg/kg) and very low concentrations in fat, plasma and erythrocytes.

Table 3. Metabolites of Piperazine-2,3-¹⁴C-enrofloxacin as a percentage of total residues in rat tissues.

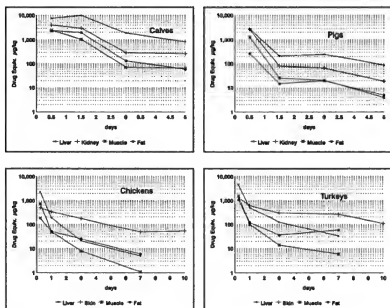
Metabolite	Liver	Kidney	Muscle
Enrofloxacin	20.2	46.6	48.5
Ciprofloxacin	4.9	13.3	17.7
Total	25.1	59.9	66.2

TISSUE RESIDUE DEPLETION STUDIES

Overview

The depletion of the total residues of 2-¹⁴C-enrofloxacin was measured in calves, pigs, chickens and turkeys. The results for edible tissues are illustrated in Figure 1 and Table 4. There were large standard deviations of the means for the residue concentration values for most tissues. The semi-logarithmic depletion was not linear during the elimination phase. However, except in the case of bovine liver the residues were below 500 µg/kg in all edible tissues by the third day after the last drug administration. The residues were most persistent in poultry skin and bovine liver and kidney tissues.

Figure 1. Total residues of ^{14}C -enrofloxacin in farm species.



Cattle

Twelve calves, body weight 31-52 kg and aged 2-4 weeks, were administered by oral gavage ^{14}C -enrofloxacin at a rate of 5 mg/kg BW for seven consecutive days. The calves were sacrificed in groups of three at 0.5, 1, 3 and 5 days after the final dose and muscle, liver, kidney and fat samples were assayed for the total radiolabeled residues (Bayer Report, 73208), see Table 4. Enrofloxacin and ciprofloxacin were the major residues.

Pigs

Ten pigs, body weight 20-25 kg, were administered by oral gavage ^{14}C -enrofloxacin at a rate of 5 mg/kg BW for seven consecutive days. The pigs were sacrificed in pairs at 0.5, 1.5, 3 and 5 days after the last dose. Muscle, liver, kidney and fat were assayed for total radiolabeled residues and the results are shown in Table 4 (Bayer Report, 73313).

Poultry

The study on chickens was done with 14 day-olds weighing 134-217 g and the study for turkeys with 21 day-olds. For each species four birds per group in five groups of birds were administered orally ^{14}C -enrofloxacin at a rate of 12 mg/kg BW for 7 consecutive days. The birds were sacrificed at 0.25, 1, 3, 7 and 10 days after the final dose. Samples of muscle, liver, fat and skin were assayed for the total radiolabeled residues (Bayer Reports, 73184 and 73185) and the results are shown in Table 4.

The skin samples were reanalysed for total radiolabeled residues and for enrofloxacin by an HPLC method (Bayer Report 73220) and the results are shown in Table 4.

Table 4. Total residues (average mean $\pm$ SD)($\mu\text{g/kg}$) in farm species after multiple oral dosing with ^{14}C -enrofloxacin.

Species & Report No Tissue	0.5 days	1.5 days	Withdrawal time 3 days	5 days	Withdrawal time 3 days	7 days	10 days
CALF 73208							
Liver	7545 $\pm$ 1631	10020 $\pm$ 4749	1908 $\pm$ 1260	838 $\pm$ 152			
Kidney	4047 $\pm$ 559	3082 $\pm$ 2476	288 $\pm$ 40	272 $\pm$ 90			
Muscle	2425 $\pm$ 769	1055 $\pm$ 751	72 $\pm$ 12	67 $\pm$ 28			
Fat	2413 $\pm$ 794	1953 $\pm$ 1690	131 $\pm$ 82	61 $\pm$ 34			
PIG 73313							
Liver	2841 $\pm$ 119	215 $\pm$ 34	250 $\pm$ 20	88 $\pm$ 10			
Kidney	2668 $\pm$ 448	81 $\pm$ 18	68 $\pm$ 35	19 $\pm$ 2			
Muscle	1295 $\pm$ 233	26 $\pm$ 8	21 $\pm$ 5	4 $\pm$ 1			
Fat	<300	<20	<20	<6			
	0.25 days	1 day	Withdrawal time 3 days	7 days			
CHICKEN 73185							
Liver	2335 $\pm$ 474	194 $\pm$ 82	29 $\pm$ 12	12 $\pm$ 4			n.m.
Muscle	748 $\pm$ 178	52 $\pm$ 16	11 $\pm$ 3	3 $\pm$ 2			n.m.
Fat	181 $\pm$ 58	56 $\pm$ 31	30 $\pm$ 33	12 $\pm$ 12			n.m.
Skin	547 $\pm$ 59	391 $\pm$ 39	214 $\pm$ 47	90 $\pm$ 37			94 $\pm$ 37
Skin 73220	466 (89%)	378 (68%)	119 (61%)	44 (50%)			
TURKEY 73184							
Liver	4713 $\pm$ 1384	513 $\pm$ 181	125 $\pm$ 49	33 $\pm$ 11			n.m.
Muscle	1573 $\pm$ 472	101 $\pm$ 16	14 $\pm$ 4	6 $\pm$ 2			n.m.
Fat	1093 $\pm$ 264	121 $\pm$ 66	36 $\pm$ 30	58 $\pm$ 37			n.m.
Skin	1360 $\pm$ 369	610 $\pm$ 298	303 $\pm$ 74	260 $\pm$ 74			111 $\pm$ 63
Skin 73220	874 (94%)	407 (85%)	210 (63%)	92 (83%)			

Each value represents the average mean of duplicate assays for 4 birds (2M, 2F). LOD 25 $\mu\text{g/kg}$ for liver and muscle, 85 $\mu\text{g/kg}$ for skin and fat based on 76 ppm (twice background).

The values for total residues in study 73220 using poultry skin are for composite samples; the values in parenthesis are the percentage of the total residues measured as enrofloxacin by HPLC.

Table 5. Residue Studies with Unlabeled Enrofloxacin.

Species	Report N°	N° animals	Tissues	Dose (mg/kg/day) °(mg/l water) [days]	Withdrawal Time (days)	LOD/LOQ (µg/kg)
Calves	12459	12	M,L,K,F	5° [3]	3, 7, 14, 21	5/?
	12459	3	M,L,K,F	2.5° [3]	7	5/?
	12459	5	M,L,K,F	5° ^{la} [3]	7, 14	5/?
	13176	12	L	5° ^{la} [5]	1, 3, 7	2/10E 5/10C
	12462	5	IS	5° ^{la} [3]	7, 14	- /10
Pigs	1148	9	IS	5° [3]	5, 7, 10	7/10
	12456	12	M,L,K,F	2.5° [3]	1, 3, 7, 14	5/20
	12456	12	M,L,K,F	2.5° ^{la} [3]	1, 3, 7, 14	5/20
	12456	12	M,L,K,F	50° [3]	3, 7, 14	5/20
Chickens	73616	6	L,Skin	25° ^m [7]	0.25, 1, 2	10/50
	73616	80	L	41° ^m [7]	0.25, 1, 1.5, 1.75, 2, 2.5, 3	10/50
	13016	16	Egg	10° [5]	1, 2, 4, 7, 10	7/10
Turkeys	None	-	-	-	-	-

° is oral dose; ^m is dose in drinking water; ^{la} is subcutaneous dose; ^f is concentration in feed, ^{la} is intramuscular injection; M is muscle, L is liver, K is kidney, F is fat, IS is injection site; E is enrofloxacin and C is ciprofloxacin

Other Residue Depletion Studies (with unlabeled drug)

The studies carried out by the sponsors are listed in Table 5.

Calves

Following the administration of enrofloxacin by either the oral route or as a subcutaneous (s.c.) injection, no residues of the parent compound were detected at 3 - 21 days after administration (Bayer Report, 12459). Ciprofloxacin was the major residue and was measured in the edible tissues by HPLC. The results of the studies outlined in Table 5 are shown in Table 6.

Table 6. Residues (µg/kg) of ciprofloxacin in calves after multiple administration of enrofloxacin

Route	Muscle	Liver	Kidney	Fat
WT (days)				
Oral				
	3	14 - 39	5	<5 - 5
	7	5 - 17	<5 - 5	<5
	14	5	<5 - 5	<5
21	<5 - 5	<5 - 5		<5
Subcutaneous				
	7	13 - 36	14 - 21	<5
14	<5	10 - 12	13 - 18	<5

WT is the withdrawal time; ENX is enrofloxacin; CIPX is ciprofloxacin

In a second study (Bayer Report, 13176) residues of enrofloxacin and ciprofloxacin were both found in livers of calves administered subcutaneous doses. In a third study (Bayer Report, 12462) residues were measured in

the neck of calves after three daily subcutaneous injections. The results of both studies are shown in Table 7.

Table 7. Residues of enrofloxacin and ciprofloxacin ($\mu\text{g/kg}$) in livers and injection sites of calves after multiple subcutaneous injection

WT (days)	Liver ENX	Liver CIPX	IS ENX	IS CIPX
1	58 - 395	325 - 614	nm	nm
3	7 - 30	37 - 68	nm	nm
7	<2 - 4	6 - 16	<10 - 12	12 - 24
14	nm	nm	<10	13 - 17

WT is the withdrawal time; ENX is enrofloxacin; CIPX is ciprofloxacin; IS is injection site; nm is not measured

Lactating dairy cows

Lactating dairy cows were administered enrofloxacin by intravenous injection daily for five days. One group of twelve cows were administered a dose of 2.5 mg/kg BW (Bayer report, V93-001) and a second group of five cows were dosed with 5 mg/kg BW (Bayer report, RA - 982/88). The morning and evening milk samples were combined to produce a daily sample for the lower dose group and were kept separate as morning and evening samples for the high dose group. The concentrations of enrofloxacin and ciprofloxacin were determined in the samples in the lower dosed cows and ciprofloxacin was measured for the higher dosed cows. The analysis was carried out using an HPLC method with an LOQ of 5 $\mu\text{g/l}$. The concentration of ciprofloxacin was higher than enrofloxacin in all the samples. The number of animals testing positive and the range of milk concentrations for both analytes is shown in Table 8.

Table 8. Residues of enrofloxacin and ciprofloxacin in milk of dairy cows following intravenous injection of enrofloxacin daily for five days

Dose/Analyte	Day 1	Day 2	Day 3	Day 4	Day 5
2.5 mg/kg bw					
Enrofloxacin (range $\mu\text{g/kg}$)	<5 - 14	<5 - 7	<5	<5	<5
Positive out of 12	10	3	0	0	0
Ciprofloxacin (range $\mu\text{g/kg}$)	7 - 49	<5 - 18	<5 - 12	<5 - 7	<5
Positive out of 12	12	6	4	2	0
5 mg/kg bw					
Enrofloxacin (range $\mu\text{g/l}$, am/pm)	<2	<2	<2	<2	nm
Ciprofloxacin (range $\mu\text{g/l}$, am)	30 - 132	5 - 23	2 - 4	<2 - 2	nm
Positive out of 5	5	5	5	3	-
Ciprofloxacin (range $\mu\text{g/l}$, pm)	8 - 39	3 - 7	<2 - 5	nm	nm
Positive out of 5	5	5	3	-	-

nm is not measured. The values between 2 and 5 are based on the LOD of 2 $\mu\text{g/l}$ and are therefore imprecise as the LOQ is 5 $\mu\text{g/l}$.

Pigs

Residues of enrofloxacin and ciprofloxacin in edible tissues were measured by HPLC after oral dosing, intramuscular injection and inclusion in the feed of enrofloxacin (see Table 5). The results are shown in table 9. The residues are low within one day of dosing and deplete rapidly so that at three days ciprofloxacin is not detected and enrofloxacin is only found in the liver and kidney tissues. No residues were detected at 7 or 14 days withdrawal time nor were any residues detected following in-feed administration (Bayer Reports 12456 & RA-1148/88).

Residues of enrofloxacin and ciprofloxacin were measured in the neck tissue (sample size between 240 and 850 g) of nine pigs after 3 daily injections in the same site (see Table 5). The results are shown in table 10. The drug was almost completely absorbed from the injection site by day 10 withdrawal time. Residues of ciprofloxacin (20 µg/kg) were detected in one pig on day 5 and one animal on day 7 (Bayer Report RA-1148/88).

Chickens

The study, Bayer Report 73616 (see Table 5), measuring residues of enrofloxacin was divided into two parts. The first part was a pilot study in which measurements for residues in liver and skin were made where the dose in the drinking water was 25 mg/l. The residues in µg/kg in skin were 220 and 140 at 6 h post dosing, 45 and 49 at 24 h and 21 and 16 at 48 h. In the main study the dose was unintentionally high at 41 mg/l water and residues were only measured in liver samples (see Table 11). There were no significant differences in the residues of males and females, thus the results are combined in the Table. The residues depleted rapidly and were <500 µg/kg two days after cessation of administration of the drug.

Table 9. Residues of enrofloxacin and ciprofloxacin (µg/kg) in pigs after multiple administration of enrofloxacin

Route WT (days)	Muscle		Liver		Kidney		Fat	
	ENX	CIPX	ENX	CIPX	ENX	CIPX	ENX	CIPX
Oral								
1	240	30	190	40	290	100	60	nd
3	nd	nd	20	nd	20	nd	nd	nd
7 & 14	nd	nd	nd	nd	nd	nd	nd	nd
Intramuscular								
1	170	30	180	40	240	70	80	nd
3	20	nd	20	nd	40	nd	nd	nd
3 & 14	nd	nd	nd	nd	nd	nd	nd	nd
Feed								
3, 7 & 14	nd	nd	nd	nd	nd	nd	nd	nd

WT is the withdrawal time; ENX is enrofloxacin; CIPX is ciprofloxacin; nd is <10 µg/kg

Table 10. Residues (µg/kg) of enrofloxacin at injection site of pigs

5 days	Withdrawal time 7 days	10 days
68400	430*	10
10900	180	nd
430*	40	nd

* these samples contained 20 µg/kg ciprofloxacin.

Table 11. Residues of enrofloxacin in livers of chickens administered 41 mg/l in drinking water

Withdrawal Time (days)	Mean n = 10 ($\mu\text{g/kg}$)	Range ($\mu\text{g/kg}$)
0.25	2610	1850 - 4860
1	870	410 - 1350
1.5	420	180 - 620
1.75	240	160 - 320
2	190	60 - 310
2.5	130	60 - 200
3	90	50 - 210

When the recommended dose of 50 mg/l in the water was administered the residues were higher and persisted for a longer period, e.g. in skin at day 4 enrofloxacin was 120 $\mu\text{g/kg}$ and ciprofloxacin was 20 $\mu\text{g/kg}$, at day 7 parent drug was 100 $\mu\text{g/kg}$ and ciprofloxacin 20 $\mu\text{g/kg}$, at day 10 enrofloxacin was 50 $\mu\text{g/kg}$ and ciprofloxacin was not detectable. Also residues of enrofloxacin but not ciprofloxacin were detected in liver of broilers for at least 17 days after dosing.

Eggs

Laying hens were administered enrofloxacin (see study 13016 in Table 5) and the residues of enrofloxacin and ciprofloxacin were measured by HPLC in egg yolk and egg white. The results are shown in Table 12. Enrofloxacin was the major residue and the levels of both compounds dropped to below the LOQ (10 $\mu\text{g/kg}$) on the tenth day after the last dose.

Table 12. Residues ($\mu\text{g/kg}$) in eggs after administration of enrofloxacin.

WT (days)	Egg White Enrofloxacin	Egg white Ciprofloxacin	Yolk Enrofloxacin	Yolk Ciprofloxacin
1	3630	200	3140	210
2	440	20	1960	250
4	40	<10	490	100
7	10	<10	70	30
10	<10	<10	<10	<10

Each value is the mean of 12-16 eggs.

METHODS OF ANALYSIS FOR RESIDUES IN TISSUES.

HPLC Methods

All of the methods submitted from the sponsor are basically the same. They are based on homogenisation and extraction of both enrofloxacin and ciprofloxacin into an organic phase. The extract may be further purified and then dissolved in the HPLC mobile phase. After resolution by HPLC, detection is by either UV or fluorescence. The earlier methods were used to measure the levels of residues in treated animals. More

recently the methods were improved by selecting better extraction solvents and changing the HPLC conditions (Bayer Reports, 93/14231-4). Samples of muscle, fat, skin or blood were extracted by homogenisation with cold ethanol/acetic acid. The extract was evaporated to dryness and the residue dissolved in the HPLC mobile phase. After clarification by centrifugation an aliquot of the solution was assayed by HPLC using fluorescence detection. The analysis of liver and kidney required a clean up step of the homogenate extract by passing it through an XAD-4 polystyrene resin. Milk samples were directly extracted into acetonitrile.

The new methods appear to meet the contemporary criteria (EC Decision 93/256). The limits of detection (LOD) are down to 1 µg/kg tissue and the limits of determination (quantification) (LOQ) are now 10 µg/kg for most tissues. Recoveries of spikes of enrofloxacin and ciprofloxacin equivalent to 10-210 µg/kg from muscle, fat, skin, liver, kidney and blood were mostly >70% except for ciprofloxacin in liver (57-59%) and kidney (68-69%) with intraassay precision well below 10% except for enrofloxacin from pig liver (10-12%) and for ciprofloxacin in fat (13-16%).

A method for the simultaneous determination of enrofloxacin and ciprofloxacin in pig muscle, bacon and bovine muscle with extraction into 1% acetic acid/ethanol and clean up on a Bond-Elut SCX column followed by HPLC with fluorescence detection is published (see Heitzman, 1994). The LOQ was 10 µg/kg.

APPRAISAL

Enrofloxacin is a member of the new group of fluoroquinolone antibiotics. The major metabolite in farm animals is ciprofloxacin which is a widely used drug in human medicine.

The drug is rapidly absorbed from the gut of calves, poultry and rats with plasma concentrations reaching peak values in less than 8 hours. 40% of an oral dose was excreted in the bile of rats. The route of excretion in farm animals was not investigated.

Metabolism was studied in farm animals and rats using ¹⁴C-radiolabeled enrofloxacin. In all the samples parent drug and ciprofloxacin were major residues except in poultry muscle and skin in which only parent drug but not ciprofloxacin was present. This is in contrast to the metabolism in the bovine where ciprofloxacin is the most identified abundant residue.

The depletion of the total residues of 2-¹⁴C-enrofloxacin was measured in calves, pigs, chickens and turkeys. There were large standard deviations of the means for the values for most tissues, also the depletion was not linear during the elimination phase. However, except in the case of bovine liver the residues were below 500 µg/kg in all tissues by the third day after the last drug administration. The residues were most persistent in poultry skin and bovine liver and kidney tissues.

The successful measurements of residues of enrofloxacin and ciprofloxacin were achieved in a single analyses by a validated HPLC method. The LOQs for edible tissues were about 10 µg/kg and 5 µg/l for milk. Residues of enrofloxacin and ciprofloxacin were measured by an HPLC method in the edible tissues, eggs and injection sites of farm animals which had been administered either orally or parentally multiple doses of the sponsors recommended doses of enrofloxacin. The residues in all tissues depleted rapidly and were either not detectable or at very low concentrations at 7 days after administration of drug.

Maximum Residue Limits

Based on the temporary ADI of 0 - 0.6 µg/kg for parent drug established by the Committee, the permitted daily intake of parent drug and/or its equivalents of antimicrobial activity is 36 µg for a 60 kg person per day.

The following factors were considered in estimating the MRLs.

1. The temporary ADI is set on a microbiological end point.
2. The limit of quantification of the method (10 µg/kg for enrofloxacin and ciprofloxacin in tissues and 5 µg/kg or l in milk).
3. The drug is for use in meat animals, poultry, lactating cows and laying hens.
4. The marker residue is the sum of enrofloxacin and ciprofloxacin for tissues. Ciprofloxacin is the marker residue for bovine milk.
5. There is an uncertain ratio of the marker residues to the total residues. Also the percentage of the total residues that possess antimicrobial activity is not known. A factor of up to 5 is probable.

The Committee noted that assuming theoretical MRLs equal to two times the LOQs for the analytical methods, the amount of marker residues in the total food basket (muscle, liver, kidney, fat, milk and eggs) would be 39 µg and just in excess of the ADI of 36 µg. However this would not take into account any antimicrobial activity of the significant fraction (up to 80% in tissues and percentage not known for milk) forming the remaining residues. It was therefore not possible to allocate MRLs which would guarantee an observance of the ADI. The Committee requests information concerning the antimicrobial activity of the remaining residues.

The following information is required for evaluation in 1997.

- studies to determine the antimicrobial activity of the residues other than enrofloxacin and ciprofloxacin.

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GENTAMICIN

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IDENTITY

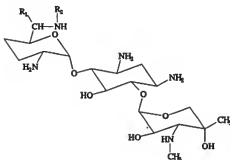
Chemical name:

Gentamicin

Synonyms:

Gentamam, Septigen, Bigental, Uterogen, Gentocin, Gentavetina
(Product Trade Names)

Structural formula:



Gentamicin C₁ :R₁ = R₂ = CH₃

Gentamicin C₂ :R₁ = CH₃ ; R₂ = H

Gentamicin C_{1a} :R₁ = R₂ = H

Molecular formula:

C₍₁₈₋₂₁₎H₃₆₋₄₁N₅O₇

Molecular weight:

475.9 (C₁, 477.6; C₂, 463.6; C_{1a}, 449.5)

OTHER INFORMATION ON IDENTITY AND PROPERTIES

Pure active ingredient:

Appearance:	White to buff-coloured powder (as sulphate)
Melting point:	218-237° (as sulphate), 102-108° (pure complex)
Solubility:	Freely soluble in water; soluble in pyridine, dimethylformamide and in acidic media with salt formation; moderately soluble in acetone, ethanol and methanol; virtually insoluble in benzene and halogenated hydrocarbons (pure complex); freely soluble in water, 0.1N hydrochloric acid and 0.1N sodium hydroxide; soluble in ethylene glycol and formamide; practically insoluble in alcohol, chloroform and ether (as sulphate).
Optical rotation:	[α] _D ²⁵ +146° (pure complex) [α] _D ²⁵ +102° (as sulphate)

RESIDUES IN FOOD AND THEIR EVALUATION

CONDITIONS OF USE

General

Gentamicin, an aminoglycoside antibiotic produced by fermentation of *Micromonospora purpurea*, is a mixture of basic, water soluble compounds containing the aminocyclitol 2-deoxystreptamine and 2 additional amino sugars. The three major active components are designated C₁, C₂ and C_{3a}, but other minor active components which may be present include gentamicins A, B, B₁ and X. The gentamicin C complex is normally formulated as the sulfate salt, a white-to-buff colored, odourless, water-soluble powder. Ion exchange procedures are frequently used for the separation and purification of gentamicin on a commercial scale, after pH adjustment of the fermentation broth and filtration. Gentamicin is indicated for the treatment of a variety of susceptible bacterial infections in swine, poultry, bovines and equines (eg., colibacillosis and peritonitis in swine; for mastitis, urinary tract disease, respiratory disease and septicemia in bovines). Gentamicin has not been previously reviewed by the Committee.

Dosage

Various formulations of gentamicin have been developed for the treatment of food producing animals, some of these in combination with other antibiotics such as penicillin G, ampicillin and cloxacillin. Formulated products typically contain from 4 - 80 mg/ml of gentamicin and are available for administration by injection, mammary infusion, oral treatment or as an additive to drinking water. Directions for use vary from one time only for the oral treatment to 1-2 injections per day for up to 3 days for injectables to 1-3 treatments for mastitis products. Typical treatment time with the drinking water additive formulation is 3 days.

Gentamicin is currently used in the following food producing animals species : swine, poultry, equine and bovine including lactating cows.

DOSAGE	VIA	TARGET SPECIES
2.5-4 mg/kg one /several treatments	oral and injection	piglets pigs
2-4 mg/kg/day	injection	1-day-old chicks
100 mg/quarter/day	infusion	lactating cows
2-5 mg/kg/day	injection	cattle
2-5 mg/kg/day	injection	equine

METABOLISM

General

Data were considered from studies of the radio-labelled drug in a beagle dog, in 3-day-old piglets and in weanling piglets. Studies with non-labelled gentamicin have also been conducted in a variety of species, including pigs, cattle and chickens. After oral administration, gentamicin is essentially non-absorbed. It is, however, well-absorbed after intramuscular or subcutaneous injection and is excreted unchanged via the kidney. In general, aminoglycosides distribute primarily to the extracellular fluid space and accumulate in the kidney (Riviere et al., 1991).

Dog

A beagle dog which received a single intravenous dose of ^{14}C -labelled gentamicin (9.4 mg/kg BW) excreted approximately two-thirds of the dose in 24-hour urine, while only traces were found in the faeces (Miller et al., 1976). The remainder of the dose was eliminated much more slowly with trace quantities present in urine 31 days post-treatment and 30% of the dose still unaccounted for. Changes in the elimination rate calculated from the urine data suggested depletion via a multi-compartment model. Consistent results were obtained for levels of gentamicin in serum collected from a second beagle which received a dose of 20 mg/kg BW (Miller et al., 1976). Three different measurement methods, total radioactivity, microbiological assay and radioimmunoassay, produced similar results which suggested that no significant metabolism occurred in the dog. Microbiological assays of urine samples provided higher results for most samples than radioactive counts, but the total gentamicin recovery for all samples tested was in good agreement. At sacrifice of the second beagle 8 days after dosing, gentamicin was detectable in all organ tissues tested by total radioactivity, with most of the residues found concentrated in the kidney cortex (approximately 400 times higher than in the skeletal muscle).

Swine

Twelve 3-month old female pigs were divided into two groups, each of which received 200 mg gentamicin oral solution or soluble powder via intubation for 3 consecutive days, in a crossover design. The estimated dose was 6.35 mg/kg BW/d, based on the average initial weights of the animals used in the study. Both formulations produced sub-therapeutic levels in plasma, with no significant difference in the concentrations attained in plasma using either formulation (Lamendola et al., 1980).

Poultry

In a study in which day-old chicks were treated with 0.2 mg gentamicin by subcutaneous injection, gentamicin was rapidly absorbed and reached peak levels in the blood within 30 min (Bickford, 1975). Rapid excretion followed, with blood levels dropping to one-half peak values within two hours. Gentamicin activity was measured in the rectum and duodenum through 24 hours, through 72 hours in the lungs and heart and to 7 days in the bile and yolk sac after this single treatment. Distribution was rapid throughout the tissues, reaching a peak level of 50.4 mg/kg in kidney 24 hours after treatment and declining to 3.3 mg/kg in kidneys at 7 days.

TISSUE RESIDUE DEPLETION STUDIES

Radiolabeled Residue Depletion Studies

Three-day-old piglets treated orally with 5 mg ^3H -gentamicin (dosage approximately 3.6 mg/kg BW/d) were sacrificed at 1, 3, 6, 11, 14 and 17 days post-treatment, using 3 treated piglets and 1 control per slaughter date (Pavelchak and Rock, 1979). Samples of liver, kidney, muscle and fat were collected and analyzed by total reactivity. The results, shown in Table 1, demonstrated that gentamicin persists for the longest period at the highest levels in kidney and showed little distribution of the drug into muscle tissue. No residues were detectable at 11 days, except in one kidney which contained 0.11 mg/kg of gentamicin.

Table 1. ^3H -gentamicin residues in swine tissues following oral administration of 5 mg to 3-day-old piglets^a, expressed as mg/kg

Days Withdrawal	Kidney	Liver	Muscle	Fat
1	4.73	0.20	<0.05	0.05 ^b
3	0.44	0.06 ^c	<0.05	<0.05
6	0.31	0.10 ^d	<0.05	<0.05
11	— ^e	<0.05	<0.05	<0.05
14	<0.05	<0.05	<0.05	<0.05
17	<0.05	<0.05	<0.05	<0.05

^a Average for three animals, 5 replicate assays per tissue per animal, assay limit of detection 0.05 mg/kg.

^b Based on samples from only one animal.

^c Average excludes samples from two animals that contained <0.05 mg/kg gentamicin.

^d Average includes one sample containing 0.11 mg/kg gentamicin.

^e Individual values <0.05, 0.11, <0.05 mg/kg gentamicin.

Gentamicin levels in kidneys from 6-week-old pigs treated intramuscularly (Rock et al., 1978 a) or orally (Rock et al., 1978 b) with ^3H -labelled gentamicin were analyzed by total radioactivity, microbiological assay and radioimmunoassay, with all assays producing similar results, thereby suggesting that the gentamicin was not significantly metabolized. In the piglets administered gentamicin, 50 mg/gallon drinking water *ad libitum*, formulated to include ^3H -labelled drug, 4.27 $\mu\text{Ci}/\text{mmole}$, were calculated to have consumed an average 5.37 ± 1.20 mg/lb (0.81 mg/kg BW/d) of gentamicin during the 3-day treatment period (Rock et al., 1978 a). Three piglets were slaughtered at each of days 1, 3, 5 and 7 post-treatment. Results of the total radioactivity assay are presented in Table 2.

Table 2. ^3H -gentamicin residues in swine tissues following administration in water (50 mg/gal) *ad libitum* for 3 days to 6-week-old piglets^a, expressed in mg/kg

Withdrawal (days)	Kidney	Liver	Muscle	Fat
1	0.18	0.11	<0.03	<0.03
3	0.06	0.08	<0.03	<0.03
5	<0.04 ^b	0.05	<0.03	<0.03
7	<0.04 ^c	0.04	<0.03	<0.03

^a Average for three animals, 5 replicate assays per tissue per animal.

^b Average includes results for one kidney that contained <0.03 mg/kg gentamicin.

^c Average includes results for two kidneys that each contained 0.04 mg/kg gentamicin.

3-Day old piglets which received a single 5 mg intramuscular injection of ^3H -gentamicin administered in the mid-region of the right semitendinosus muscle were sacrificed at 14, 28, 35, 42 or 49 days after medication (Rock et al., 1978 b). Samples of kidney, liver, fat, muscle and injection site were analyzed by the three methods described in the previous experiment. Again, the close agreement of the three assay methods used suggested no significant metabolism, as the presence of metabolites should lead to differences in the analytical results obtained with the different assay methods. Highest concentrations of gentamicin were found in kidney for all collection dates and animals tested, as shown in Table 3.

Table 3. ^3H -gentamicin residues (mg/kg) in swine tissues following administration of a single intramuscular injection (5 mg) to 3-day-old piglets*

Withdrawal (d)	n	Kidney	Liver	Inj. Site	Muscle	Fat
14	2	0.68	0.42	0.12	<0.02	<0.02
28	3	0.18	0.11	<0.02	<0.02	<0.02
35	3	0.07	0.06	<0.02	<0.02	<0.02
42	3	0.05	0.04	<0.02	<0.02	<0.02
49	1	0.02	0.02	<0.02	<0.02	<0.02

* Based on five replicate assays per tissue sample

Other Residue Depletion Studies (with Unlabeled Drug)

Cattle

Gentamicin was administered to recently weaned calves at 4 mg/kg BW/day by IM injection in the neck on three successive days (Banting, 1962 a). The calves were divided into groups of 3-5 which were slaughtered, respectively, at 7, 30, 60, 70 and 80 days after the final treatment. Muscle, liver and kidney samples were collected for all dates, and injection site samples were collected from the 30-day and 60-day groups. The samples were analyzed using a bioassay with a limit of detection of 0.05 mg/kg for gentamicin. Residues were present in all kidney samples tested, ranging from >10 mg/kg at day 7 to 0.45-0.75 mg/kg at day 80. Detectable residues were present in 2 of 3 liver samples at day 70 (0.35, 0.50 mg/kg) and in one of 3 liver samples from day 80 (0.10 mg/kg). One muscle sample from day 7 contained 3.6 mg/kg of gentamicin, while all other muscle samples, including those from injection sites, contained no detectable residues. This experiment was confounded by illness of the calves and the death of seven of the experimental animals. Results of this study are summarized in Table 4.

Table 4. Gentamicin residues in calf tissues as determined by cylinder plate assay following intramuscular administration of a single dose of 4 mg/kg BW, expressed as mg/kg*.

Withdrawal (d)	n	Kidney	Liver	Inj. Site	Muscle
7	5	>10	3.6	na ^b	nd ^c
30	3	2.0	0.8	nd	nd
60	5	1.1	0.6	nd	nd
70	3	0.9	0.3	na	nd
80	3	0.6	0.03	na	nd

* Assay limit of detection 0.05 mg/kg

^b na = not analyzed

^c nd = not detected

Twenty weaned calves were treated with 4 mg/kg BW by both intramuscular and oral dose, followed 12 hours later by an additional oral dose of 4 mg/kg BW (Pavelchak and Lamendola, 1980 a). Three calves and one control were slaughtered at each of 70, 80, 90 and 100 days of withdrawal and samples of kidney, liver, muscle and fat were collected. Only kidney tissue was analyzed. At 70 days, average residues in kidneys, determined by radioimmunoassay, were 0.48 ± 0.25 mg/kg and averaged less than 0.3 mg/kg at other sampling dates. Kidney samples from one animal killed at 100 days, which was consistently ill and had poor weight gain during the experiment, contained 0.47 mg/kg gentamicin. This result may be more representative of the persistence of residues in sick animals than the residues observed in the healthy animals in the study.

Five mature dairy cows were also treated with gentamicin, at 4 mg/kg BW/day, for three successive days, by IM injection in the neck, using several injection sites per treatment (Banting et al., 1982 b). Milk samples were collected and analyzed as above at 24, 38, 46, 60, 68, 82 and 90 hours following the last treatment. One sample of milk taken at 46 hours contained 0.07 mg/kg gentamicin, while all others were negative showing results below the detection limit, 0.05 mg/kg.

Three studies report residues resulting from the administration of gentamicin by intramammary infusion (Sprent and Riggs, 1978; Carver et al., 1981; Sprent and Lobell, 1980). Five normal mature lactating cows received 100 mg of gentamicin in each quarter immediately after each of three successive milkings (Sprent and Riggs, 1978). Samples were analyzed by a microbiological cylinder-plate assay and 96-hour sample results were confirmed by radioimmunoassay, with highest results found in samples taken during and twelve hours after the treatment period. The average concentration of gentamicin in the milk samples was <0.02 µg/ml in the 72-hour samples and remained in this range in subsequent samples to 144 hours post-treatment.

Four cows (Holstein, age 4-5 years) each received 200 mg gentamicin (Gentocin) by intramammary infusion in the right front quarter, after which milk was collected at 12-hour intervals to 120 hours post-treatment (Carver et al., 1981). Radioimmunoassay results indicated an average level of 0.08 µg/ml gentamicin at 48 hours, reduced to 0.02 µg/ml at 60 hours and not detectable after 84 hours.

Five normal lactating Holstein cows each received 50 mg of gentamicin sulfate and 100,000 IU of procaine penicillin G by intramammary infusion in each quarter for each of three successive milkings, with a 12 hour spacing between milkings (Sprent and Lobell, 1980 a). Results of the analysis of milk samples from this study (Sprent and Lobell, 1980 b) are given in Table 5. Liver, muscle and fat samples collected from animals slaughtered at 10 and 20 days post-treatment contained no detectable gentamicin residues. Residues in kidneys were 0.57 and 0.81 mg/kg, respectively, in the two animals slaughtered at 20 days, and 0.42, 0.25 and 0.36 mg/kg in the kidneys from the three animals slaughtered 30 days after treatment.

Table 5. Gentamicin residues in milk (mg/l) collected from normal lactating cows following administration of a 10 ml intramammary infusion of Mastogen (50 mg gentamicin sulphate, 100,000 IU procaine penicillin G) per quarter at each of three successive milkings

Hours After Final Treatment	n	Cow 1	Cow 2	Cow 3	Cow 4	Cow 5
12	4	2.30 ± 0.21	2.63 ± 0.96	1.21 ± 0.34	2.84 ± 0.49	1.63 ± 1.08
24	4	0.25 ± 0.02	0.25 ± 0.08	0.26 ± 0.09	0.61 ± 0.19	0.16 ± 0.08
36	4	<0.05	0.06	<0.05	0.10	<0.05
48	4	nd	<0.05	nd	<0.05	<0.05
60	4	nd	nd	nd	nd	<0.05
72	4	nd	nd	nd	nd	nd

nd means not detected; <0.05 means milk from one or more quarters tested positive at or slightly above the assay limit of detection, but the pooled sample for the four quarters should not contain detectable residues.

Four reports were presented on experiments where gentamicin was administered by intrauterine infusion. In each of the first two of these experiments, three mature cows received 200 mg of gentamicin by intrauterine infusion and were slaughtered after withdrawal periods of 15 days (Loy, 1973 a) and 20 days (Loy, 1973 b), respectively, with collection and analysis of kidney, liver, muscle and fat. At 15 days, two of three animals contained detectable gentamicin residues in their kidneys (0.11 and 0.13 mg/kg) (Loy, 1973 a), while at 20 days the kidneys from one of the three animals contained 0.38 mg/kg of gentamicin (Loy, 1973 b). No other tissues collected from the 15 or 20 day animals contained detectable residues of gentamicin, using a microbiological assay.

In a similar study, 5 Holstein cows in early or mid-lactation, 4-5 years of age, received 200 mg of gentamicin by intrauterine infusion and were slaughtered 30 days after treatment (Bickford, 1974). No milk samples collected (6-94 hours post-treatment) contained any detectable gentamicin residues, using a microbiological assay with a limit of detection of 0.01 µg/ml. No detectable residues were found in liver, kidney, muscle or fat samples collected after 30 days withdrawal. The assay limit of detection ranged from 0.04 mg/kg in kidney and fat to 0.16 mg/kg in liver.

Finally, 9 Holstein cows (3-year old) were treated with 200 mg gentamicin by intrauterine infusion and 3 animals were slaughtered at each of 7, 15 and 30 days post-treatment. Milk samples were collected at 12-hour intervals for 5 days (Pavelchak and Lamendola, 1980 b). A tenth Holstein served as a control. All samples were analyzed by radioimmunoassay, with a limit of detection of 0.01 ppm in both milk and tissue samples. No milk samples contained detectable gentamicin residues, nor did muscle, liver or fat samples. Residues in kidney tissues averaged 0.121 mg/kg at day 7, 0.017 mg/kg at day 15 and were only detectable in kidneys from one of three animals at day 30 (0.010 mg/kg). The results of the four experiments were consistent.

Swine

Experiments were conducted using non-radiolabelled gentamicin in the same formulation with healthy (Pavelchak and Lamendola, 1980 c) and colibacillosis-infected (Pavelchak et al., 1980) 3-day-old piglets. Healthy three-day-old piglets which received a single oral dosage of 5 mg of gentamicin (approx. 2.56 mg/kg BW) were sacrificed at 1, 3, 6, 9, 11 and 14 days post-treatment (3-5 piglets per slaughter date) [6]. Average gentamicin residues in kidneys as determined by radioimmunoassay declined from 1.29 mg/kg on day 1 to 0.74 mg/kg at day 6 and 0.04 mg/kg at day 14.

Infected 3-day old piglets treated with a single oral dose of 5 mg of gentamicin (approx. 3.68 mg/kg BW) were slaughtered in groups of three at each of days 1, 3, 6 and 11 post-treatment and kidneys were analyzed by radioimmunoassay (Pavelchak et al., 1980). Average gentamicin residues found in kidneys were: day 1, 0.96 ± 0.54 mg/kg; day 3, 1.08 ± 0.70 mg/kg; day 6, 0.35 ± 0.05 mg/kg; day 11, 0.07 ± 0.02 mg/kg. The report provided no results for other tissues.

In a related experiment, twenty-six 3-day-old piglets were treated with 5 mg gentamicin orally (estimated dose 3.25 mg/kg BW). After treatments, piglets were assigned randomly into six groups composed by 3 or 5 animals and slaughtered at 1, 3, 6, 9, 11 and 14 days post-treatment. Kidney, liver, muscle, and fat were collected and analyzed by a microbiological assay with a limit of detection of 0.04-0.08 mg/kg, depending on the tissue. No detectable residues were found in any muscle or fat samples, one liver contained 0.4 mg/kg gentamicin at day 3 and residues in kidneys were below the detection limit, 0.08 mg/kg, at day 14. (Lobell et al, 1980).

Poultry

In 1-day-old chicks treated with 0.2 mg gentamicin by subcutaneous injection (Bickford, 1975), the highest gentamicin residues observed in liver, skin/fat and muscle were in the range of 4.0-4.4 mg/kg within 3 hours of treatment. At 7 days post-treatment, no gentamicin was detectable in muscle, while residues detected in fat/skin and liver were 0.1 and 1.1 mg/kg, respectively.

METHODS OF ANALYSIS FOR RESIDUES IN TISSUES AND MILK

General

A radioimmunoassay specific to gentamicin with a claimed limit of detection of 0.02 mg/kg has been described for the analysis of porcine kidneys (Rock and Lamendola, 1978) and tested against an antimicrobial cylinder plate assay and total recovery of radio-labelled gentamicin (Rock et al., 1978 a, b). Results of a direct comparison of these two methods with gentamicin residues as determined by total radioactivity is given in Table 6.

Table 6. Gentamicin residues in kidneys from piglets treated orally with 5 mg gentamicin, assayed by different methods			
Days	Gentamicin	(mg/kg)	
Withdrawal	Microbiological	RIA	Radiotracer
14	0.61	0.67	0.68
28	0.12	0.20	0.18
35	<0.08	0.07	0.07
42	<0.08	0.05	0.05
49	<0.08	0.02	0.02

In the radioimmunoassay method, tissues were homogenized with 1.0N H₂SO₄, centrifuged and the pellet was re-extracted with water. The combined supernatants were adjusted to pH 7.4 and again centrifuged, after which the supernatant was diluted to 100 ml with Tris buffer and assayed.

Details of a microbiologically based cylinder plate assay system for gentamicin have also been described (Rock et al., 1978 a). Samples were prepared for assay using extraction procedures similar to those described above. Petri dishes were prepared with a pre-seeded agar medium containing an 0.1% suspension of *S. epidermidis* ATCC 12228 over an unseeded base layer. Assay dishes stood at room temperature for one-half hour prior to sample addition. Six stainless steel cylinders were then placed on the surface of the agar and three were alternately filled with standard or test samples, while the remainder were filled with reference solution. After 1 h pre-incubation at room temperature, the assay dishes were incubated overnight at 35°. Concentrations were estimated from the diameters of the zones of growth inhibition. The test has a claimed limit of detection of 0.08 mg/kg for gentamicin residues in porcine kidney. Details of the selectivity of this test against other antibiotics were not provided.

Other assay methods for gentamicin residues in animal-derived foods that have been described include thin layer chromatography (TLC) (Lagner and Teufel, 1972) and TLC-bioautography (Yoshimura et al., 1982; Salisbury et al., 1989). None of these TLC-based methods appear sufficiently sensitive for routine regulatory use. Extraction methods for quantitation of gentamicin residues in kidney and muscle using a commercially available ELISA have been reported, with the additional application of the method to gentamicin in milk (Brown et al., 1990). Among the gentamicin-specific test kit technologies that are commercially available are receptor assays and a variety of kits using ELISA technology in well or card formats. While independent testing has not been done on all these tests in all matrices of interest, there does appear to be a selection of commercially available test kit technologies suitable for use in regulatory screening programs for gentamicin.

The determination of gentamicin in kidney using ion-pairing liquid chromatography (LC) followed by post-column derivatization with o-phthalaldehyde (OPA) has been reported, but no detection limit was given (Lacharte et al., 1983). A similar approach with pre-column derivatization with OPA for the determination of gentamicin in muscle, with a detection limit of 0.2 mg/kg and recoveries of 69-105% has also been described (Agarwal, 1989). An LC method for the qualitative identification of gentamicin residues in porcine tissue at levels above 0.4 mg/kg has also been identified (FSIS, 1991). It is not clear that any of these methods are suitable for routine use in a regulatory laboratory.

More recently, methodology for the quantitative determination of gentamicin residues in muscle, liver, kidney and fat from swine, sheep and cattle has been included in a publication prepared on behalf of the Commission of the European Communities (Heitzman, 1994). This methodology references an earlier publication (Sar et al., 1993). The principal steps of the method include homogenization, protein precipitation using 5% trichloroacetic acid, clean up on an ion exchange column using CM-Sephadex, then high performance liquid chromatography on an end-capped 5 μ RP-18 column at 45°, followed by post-column derivatization with OPA. The method has a claimed limit of quantitation of 0.05 mg/kg for fat and muscle and 0.10 mg/kg for liver and kidney, with reported recoveries in muscle of 74% at 0.8 mg/kg and 88% at 0.1 mg/kg. Recoveries are in the same range for other tissues.

No data have been published on inter-laboratory trials.

A multi-residue confirmation method for aminoglycoside antibiotics in bovine kidney using ion spray LC/MS/MS, applicable to four components of gentamicin (C_1 , C_{1a} , C_2 and C_{2a}), has recently been reported (McLaughlin et al., 1994). The method includes clean-up by matrix solid phase dispersion, followed by separation using a gradient LC system. Three ions were monitored for each of the gentamicin components and the method was tested successfully on incurred samples. Gentamicin components were recovered at about 60% and minimum detectable levels for each of the four components were <0.10 mg/kg. The method appears suitable as a confirmatory procedure for laboratories which have the required equipment.

APPRAISAL

Studies in several species indicated that there is little absorption of gentamicin into edible tissues, other than kidney, for most forms of drug administration. However, little information was available concerning the persistence of gentamicin residues at injection sites (2 studies, 2 and 5 samples, respectively). Radiolabeled residue depletion studies were conducted only in piglets. These and other residue depletion studies done with swine and cattle showed that residues were most persistent from intramammary injection and this method of administration also produced persistent detectable levels in liver. No significant persistent residues of gentamicin were found in muscle samples in any of the studies conducted. Residues also cleared from milk within a week of administration of gentamicin by intramammary infusion or intramuscular injection. Treatment of dairy cattle by intrauterine infusion produced no detectable gentamicin residues in milk at any subsequent milkings, but residues were detectable in kidneys for up to 30 days post-treatment. Based on these studies, the kidney is the appropriate target tissue for residue monitoring. The studies also showed little indication of metabolism based on the close agreement of results found by three different assay methods in several studies, so gentamicin parent compound(s) should be the appropriate marker residue. It was noted that there are three or four significant components in gentamicin which should be detected in a determinative or confirmatory analytical method.

The single study reported for poultry in which 1-day-old chicks were injected subcutaneously with gentamicin was insufficient to assess the potential for persistence of gentamicin in market weight broiler chickens or in eggs which might result from this treatment. Residues in excess of 1 mg/kg were detectable in kidney and liver samples at 7 days post-treatment.

The status of current residue methodology, in brief, is that there are available commercial test kit technologies suitable for the screening of milk and tissue samples for gentamicin residues and assays based on microbial growth inhibition are available for laboratory determination of these residues. There is, however, a need for a validated chemical assay for the determination of gentamicin residues in milk. While no multi-laboratory method validations have been reported, one recently reported LC method meets the performance criteria of the Codex Committee on Residues of Veterinary Drugs in Foods. A recently published LC/MS/MS method should be suitable for confirmation of gentamicin residues in tissue samples, but the equipment is not currently routinely available in all regulatory laboratories. Based on currently published methodologies, limits of quantitation and confirmation in the range of 0.1 mg/kg appear feasible.

Maximum Residue Limits

Based on the temporary ADI of 0.4 μ g/kg body weight established by the Committee using a microbiological

end point, the permitted daily intake of gentamicin would be 240 µg of antimicrobially active gentamicin residues contributed by 500 g of food-animal tissues together with 1.5 l of milk in the diet of a 60-kg person. This was expressed as parent drug as there was no indication of significant metabolism. The Committee recommended temporary MRLs for gentamicin of 100 µg/kg for muscle and fat, 200 µg/kg for liver, and 1000 µg/kg for kidney in both cattle and pigs, as well as 100 µg/l for cattle milk, expressed as parent drug.

The temporary MRL allocated to milk takes into account the limit of quantification of current analytical methods. No MRLs were assigned to poultry or eggs because appropriate data were not available. The temporary MRLs recommended above would result in a daily maximum intake of 255 µg of gentamicin residues based on a daily intake of 300 g of muscle, 100 g of liver, 50 g of kidney, 50 g of fat, and 1.5 l of milk.

The following information is required for evaluation in 1997:

- a validated chemical analytical method with a limit of quantification at or preferably below the MRL recommended for milk.

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NEOMYCIN

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IDENTITY

Chemical name: Neomycin sulfate

Classification: Aminoglycoside antibiotic

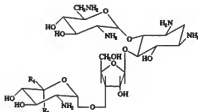
Chemical Abstracts
Service Name:

D-Streptamine, O-2,6-diamino-2,6-dideoxy-.alpha.-D-glucopyranosyl-(1→4)-O-[O-2,6-diamino-2,6-dideoxy-.beta.-L-idopyranosyl-(1→3)-.beta.-D-ribofuranosyl-(1→5)]-2-deoxy-, sulfate (1:1)(salt)

Chemical Abstracts
Registry Number:

25389-98-4

Structural formula:



Neomycin B: $R_1 = H$ and $R_2 = CH_2NH_2$

Neomycin C: $R_1 = CH_2NH_2$ and $R_2 = H$

Molecular formula: $C_{20}H_{46}N_{10}O_{13}H_2O_8S$

Molecular weight: 712.73

OTHER INFORMATION ON IDENTITY AND PROPERTIES

Pure active ingredient: Neomycin B Sulfate

BP and EP Specification:	Not less than 680 $\mu\text{g}/\text{mg}$
USP Specification:	Not less than 600 $\mu\text{g}/\text{mg}$
Agriculture grade:	571 $\mu\text{g}/\text{mg}$
Technical grade:	571 $\mu\text{g}/\text{mg}$

Impurities:	BP and EP specifications:	Neomycin C: 3-15 %
		Neamine: Not more than 2 %

Appearance: White or off-white amorphous powder

Optical Rotation:	BP and EP Specifications:	+53.5 - +59.0°
pH:	BP and EP Specifications:	5.0 - 7.5
Solubility:	mg/mL at 28°C	
	Water	6.3
	Methanol	0.225
	Ethanol	0.095
	Isopropanol	0.082
	Isoamyl alcohol	0.247
	cyclohexane	0.08
	benzene	0.05

RESIDUES IN FOOD AND THEIR EVALUATION

CONDITIONS OF USE

General

Neomycin has been used for more than 40 years as a human therapeutic for bacterial gastro-intestinal infections. The sulfate salt of neomycin has been in constant use for over 35 years on a worldwide basis for combating bacterial gastro-intestinal infections in cattle, sheep, goats, swine and poultry. Neomycin sulfate is also used in intramammary infusions to treat mastitis. Various formulations include premixes, water soluble products and intramammary infusions. Its spectrum of activity includes many gram-negative and gram-positive organisms.

Dosages

A dose of 10 mg neomycin sulfate/kg body weight is equivalent to 7.0 mg neomycin base/kg body weight. The following dose ranges are expressed as neomycin sulfate.

Cattle:	10-22 mg/kg
Cows (Intramammary):	150 to 350 mg/infusion
Sheep:	10 mg/kg
Swine:	10-22 mg/kg
Poultry:	10-30 mg/kg (chickens, turkeys, ducks)

The duration of administration of neomycin sulfate is 3-7 days for poultry and up to 14 days for larger animals. Typical durations for dairy cows and swine are 5 and 10-14 days, respectively.

METABOLISM

Pharmacokinetics

Neomycin is poorly absorbed from the intestinal tract in man and animals (3 to 10%) and has low absorption from the udder. Absorbed neomycin collects in the kidney and, to a lesser extent, in the liver. It is not metabolized except by phosphorylation, adenylation, and acetylation in the digestive tract: absorbed neomycin remains as parent compound. Orally administered neomycin is excreted primarily in the feces (>90%). Absorbed neomycin is excreted via the kidneys by glomerular filtration. After intramammary administration, neomycin is "flushed" from the udder by milking (in the dry cow after freshening).

Rat

In studies with rats given ^{14}C -neomycin in their feed, biliary and urinary excretion of radioactivity were both very low (0.27 ± 0.01 and $0.83 \pm 0.07\%$) of the ^{14}C in the dose, respectively, $n = 3$). This excretion data along with data on the ^{14}C -residues of tissues from calves indicates that urinary excretion is an approximation of the absorption of neomycin. (Aschbacher, 1994)

Cattle

Eleven calves of various ages were dosed with ^{14}C -labeled neomycin in a study to examine the influences of age, diet, and method of administration on the disposition of neomycin. All calves were killed 96 h after a single oral dose of approximately 30 mg/kg body weight. As indicated by urinary excretion, absorption of neomycin was greater in 3 day old calves (11.1 %) than in 54- to 64-day old non-ruminating calves (1.5 %) dosed similarly. Absorption of neomycin was similar in non ruminating (0.58 %) and ruminating (2.13 %) calves when the doses were administered in solution via a nipples bottle. In ruminating calves, absorption was somewhat less when the dose was administered on feed via a gelatin capsule (0.5 %) than when given in solution via a nipples bottle (2.13 %). Concerning the level of absorption of neomycin, this study indicates that the age of the calves is more important than whether the calves are ruminating or not. The concentrations of neomycin in muscle, liver and kidney are given in Table 1. (Aschbacher, 1994)

Table 1. ^{14}C Concentrations (expressed as equivalents of neomycin on a fresh tissue basis) in tissues 96 hours after oral dosing of calves with ^{14}C -neomycin

Age at dosing, d	Neomycin equivalent, $\mu\text{g/g}$		
	Kidney	Liver	Muscle
3 (n = 2) ^a	55.0 $\pm$ 14.9	1.93 $\pm$ 0.49	0.091 $\pm$ 0.007
12 (n = 1) ^a	24.0	0.67	0.044
54-64 (n = 3) ^a	4.9 $\pm$ 2.85	0.17 $\pm$ 0.06	0.016 $\pm$ 0.007
53-63 (n = 3) ^{a,b}	7.4 $\pm$ 3.40	0.33 $\pm$ 0.11	0.064 $\pm$ 0.070
59-60 (n = 2) ^{a,c}	0.77 $\pm$.73	0.11 $\pm$ 0.08	0.024 $\pm$ 0.002

^aDose administered in solution via a nipples bottle.

^bCalves had fully developed rumen at time of dosing.

^cDose administered on ground grain via a gelatin capsule.

Six ruminating dairy calves, approximately 30 months old, were given neomycin sulfate orally (overdosed at 96 mg/kg, b.i.d.) for fifteen and one half days. The mean absorption was small (0.45 %) with a wide range (0.01-1.27 %). (Podersoli, 1994).

Poultry

Neomycin, in a single dose of 30 mg/kg body weight, was given in drinking water to fasted broilers. Blood level reached 2.02 $\mu\text{g/ml}$ 30 minutes after medication. Two hours after, levels in 1 g or 1 ml of tissues were 32.77 μg in the small intestine wall, 8.35 μg in kidney, 2.12 μg in the lung, 1.36 μg in plasma, 0.66 μg in the liver and 0.49 μg in the heart. (Ibayashi, 1994).

Metabolism in Food and Laboratory Animals

Cattle

Greater than 90% of the radioactivity extracted from the kidneys of calves dosed with ^{14}C -neomycin was estimated to be in the form of neomycin. A bioassay of the kidney yielded a value of 30 $\mu\text{g/g}$ as compared to the radio assay of 45 $\mu\text{g/g}$ neomycin equivalents. This is good agreement considering that the extraction procedure of the bioassay may not have removed all of the neomycin. This study confirms the fact that neomycin is not metabolized (Aschbacher, 1994).

TISSUE RESIDUE DEPLETION STUDIES

Cattle

Twenty cattle (10 steers and 10 heifers) approximately 6 months of age were placed on a 14-day treatment of medicated water calculated to provide approximately 10 mg neomycin sulfate per pound body weight (22 mg/kg) daily. Two untreated, negative control cattle (1 steer, 1 heifer) were housed in a separate pen for the treatment phase. After the medication period, 4 treated cattle (2 of each gender) were killed for tissue collection and drug residue analyses at each withdrawal interval of 0 hours and at 1, 3, 7 and 14 days (only 3 cattle, however, were sampled on withdrawal day 7 due to a bloat mortality of a heifer midway through the treatment phase). Both unmedicated control cattle were also killed on the last medication day and their tissues assayed for neomycin. Tissues were assayed for neomycin residues by using a cylinder-plate microbiological method.

No neomycin was detected in tissues from the 2 unmedicated control cattle. Neither were neomycin residues found in the muscle, liver or kidney fat tissues of any of the medicated cattle at any sampling point. Neomycin residues were only found in the kidneys of treated cattle. Kidney neomycin concentrations were similar at zero and 24 hours withdrawal, at 0 hours withdrawal the mean level was 2.791 $\mu\text{g/g}$ and it was 2.899 $\mu\text{g/g}$ at 24 hours withdrawal. At 3 days withdrawal, the mean kidney neomycin level was 1.685 $\mu\text{g/g}$. By 7 days withdrawal, 2 of the 3 treated cattle that were sampled had detectable kidney neomycin residues below the 0.5 $\mu\text{g/g}$ limit of quantification (LOQ) and 1 animal had a level of 0.62 $\mu\text{g/g}$. One of the 4 treated cattle sampled at 14 days withdrawal had residues below the 0.5 $\mu\text{g/g}$ LOQ and the other 3 treated cattle sampled at 14 days withdrawal did not have detectable residues. Concentrations of neomycin in kidneys of cattle are shown in Table 2 (Ronning, 1993).

Table 2. Kidney tissue neomycin residue levels ($\mu\text{g/g}$) in cattle, sheep, goats and swine.

Withdrawal		Assayed neomycin levels ($\mu\text{g/g}$)		
Time, d	Cattle	Sheep	Goats	Swine
0 (12 h for	2.912		1.752	1.464
0 goats)	3.436		0.503	1.806
0	2.607		1.324	1.602
0	2.209		<0.5	3.824
Mean	2.791		1.020	2.174
1	2.209	0.537	1.891	1.338
1	2.536	0.707	1.165	<0.5
1	4.169	1.802	3.313	2.833
1	2.680	0.881	2.041	3.008
Mean	2.899	0.982	2.103	1.920
3 (2 day for	1.723	ND	1.891	<0.5
3 goats)	1.272	ND	0.556	1.084
3	1.924	0.522	2.203	1.651
3	1.821	<0.5	2.203	0.595
Mean	1.685		1.713	0.958
7 (3 day for	0.620	ND	<0.5	ND
7 goats)	<0.5	ND	1.542	<0.5
7	**	ND	0.516	0.991
7	<0.5	ND	1.843	<0.5
Mean			1.100	0.498
14 (4 day for	ND***	ND	ND	ND
14 goats)	ND	ND	1.000	ND
14	ND	ND	1.290	ND
14	<0.5	ND	0.503	0.906
Mean			0.698	0.227
21		ND		
21		ND		
21		ND		
21		ND		

*Values reported of <0.5 are for results that were below the analytical lower limit of quantification of 0.5 $\mu\text{g/g}$.

**No sample available, animal died.

***None Detected; i. e., no measurable zone of inhibition.

Six calves (3 bulls, 3 heifers) weighing 275-400 lbs, approximately 4 months old, received an oral dose of 41.8 mg neomycin sulfate (29.3 mg neomycin base)/kg body weight/day for 14 days. Two calves (1 bull, 1 heifer) served as controls. At 12 hours past the last treatment, all calves were euthanized and the kidneys collected for analysis.

Kidney samples were analyzed by microbiological assay using the method of Barbiers and Neff. Standards were prepared in extracts of control tissue because of what the sponsor described as a positive bias when neomycin was combined with tissue. At zero withdrawal, the mean concentration of neomycin in the kidney was 16.6 $\mu\text{g/g}$, range 10.5-21.0 $\mu\text{g/g}$ (Stahl, et. al., 1989).

Six non-ruminating calves, 2-4 days of age and average weight 43 kg, were dosed with neomycin sulfate to give 18.6 mg neomycin base/kg/day for 14 days. The calves were sacrificed at 12 hours following the final dose, and the kidneys were collected for neomycin analysis. Neomycin was not detected in the kidneys of the control calves at detection limits of 0.27 $\mu\text{g/g}$, but in medicated calves, the average concentration was 71.1 $\mu\text{g/g}$ (Fagerberg, 1988).

The kidneys of the calves in the above study were analyzed for neomycin by both microbiological assays and an HPLC assay. The average of the total neomycin (neomycin B plus C) by the HPLC method was 303 $\mu\text{g/g}$. Average neomycin levels of 161 and 400 $\mu\text{g/g}$ were determined using both a buffer-based and a spiked-tissue-based standard curves, respectively. A second laboratory determined the neomycin levels in one of the animals to be 296 $\mu\text{g/g}$ (Shaikh, et. al., in press).

Sixteen, 3 day old, non-ruminating Holstein bull calves were given a dose of 10 mg neomycin sulfate /lb (15.4 mg neomycin base/kg) body weight, added to a milk replacer. The dose was administered once daily for fourteen days. Groups of four calves each were slaughtered at 7, 14, 21, and 28 days after the last dose, and the kidneys assayed for neomycin by microbiological methods (Arnold, 1990).

Cattle - Dairy

Five Holstein cows were orally dosed with 15.4 mg neomycin base/kg body weight/day for four days. Milk samples were collected beginning with an initial sample taken before treatment through 18 milkings (12 hour intervals for 9 days). The limit of detection for the assay was 0.2 $\mu\text{g/g}$. Neomycin was not detected in any of the milk samples during the first 6 days of the study and the analysis was concluded at that point (Van Buren, 1986).

Sheep

Twenty sheep (10 wethers and 10 ewes) were placed on a 14-day treatment program of neomycin drench solution administration s.i.d to provide approximately 10 mg neomycin sulfate per pound body weight. Two untreated, negative control sheep (1 wether, 1 ewe) were housed in a separate pen during the treatment phase.

After the medication period, 4 treated sheep (2 of each gender) were killed for tissue collection and drug residue analyses at each withdrawal interval of 1, 3, 7, 14 and 21 days. Just prior to the first withdrawal phase tissue collection from medicated sheep, tissues were collected from 2 unmedicated control sheep that were also included in the study. Collected tissues were assayed for neomycin residues using a cylinder-plate microbiological method.

Neomycin was not detected in any tissue from the 2 unmedicated control sheep. Neither was neomycin found in muscle, liver or fat tissues of any medicated sheep at any sampling point. Neomycin was found only in kidneys of treated sheep. All 4 sheep sampled at 24 hr withdrawal were positive for kidney neomycin residues, levels ranged from 0.537 to 1.802 $\mu\text{g/g}$ and averaged 0.982 $\mu\text{g/g}$. Of tissues collected at 3 days withdrawal, only 1 of 4 animals sampled had a quantifiable kidney neomycin concentration (0.522 $\mu\text{g/g}$). Another 3-day withdrawal sheep had detectable (<0.5 $\mu\text{g/g}$) kidney residues, and the remaining two 3-day withdrawal sheep had no detectable residues. Kidneys collected at withdrawal days 7, 14 and 21 did not have detectable neomycin. Concentrations of neomycin in kidneys of sheep are shown in Table 2 (Ronning, 1994).

Goats

Twenty goats, 10 castrated males and 10 females, were treated 14 days with neomycin drench solution administered orally s.i.d to provide approximately 10 mg neomycin sulfate per pound body weight. Two untreated, negative control goats (1 of each gender) were housed in a separate pen during the treatment phase.

After the medication period, 4 treated goats (2 of each gender) were killed for tissue collection and drug residue analyses at each withdrawal interval of 12, 24, 48, 72 and 96 hours. Tissues were collected from both unmedicated control goats just prior to initial tissue collections from medicated goats. Collected tissues were assayed for neomycin residues using a cylinder-plate microbiological method.

Neomycin was not detected in any tissue from either of the 2 unmedicated control goats. Neither was neomycin found in muscle, liver or fat tissues of any medicated goats at any sampling point. Neomycin was found only in kidneys of treated goats; levels averaged approximately 1.0 $\mu\text{g/g}$ at 12 h withdrawal, 2.1 $\mu\text{g/g}$ at 24 h withdrawal, 1.7 $\mu\text{g/g}$ at 48 h withdrawal, 1.1 $\mu\text{g/g}$ at 72 h withdrawal and 0.7 $\mu\text{g/g}$ at 96 h withdrawal. Concentrations of neomycin in kidneys of goats are shown in Table 2 (Roening, 1994b).

Swine

Twenty pigs, ten barrows and ten gilts, were treated 14 consecutive days with medicated water calculated to provide approximately 10 mg of neomycin sulfate per pound of body weight daily. The 20 treated pigs were housed in 5 groups of 4 pigs each, with each group of pigs in an individual, raised crate located in a climate-controlled indoor facility. Similarly, the two untreated control pigs, one barrow and one gilt, were housed in the same building in an individual, raised crate.

At the conclusion of the medication period, four treated pigs (two of each gender) were euthanized for tissue collection and drug residue analyses at each of the withdrawal intervals of 0 hours and at 1, 3, 7 and 14 days. Both unmedicated control pigs were also killed on the last medication day (withdrawal interval 0), and their tissues assayed for neomycin. Tissues were assayed for neomycin residues by using a cylinder-plate microbiological method.

No neomycin was detected in muscle, fat or liver tissues from any treated or control pig. For the kidney tissues of the two non-medicated control pigs, one pig had no detectable neomycin residues and one pig had an assayed neomycin level below the limit of quantification ($<0.5 \mu\text{g/g}$). Mean assayed kidney neomycin level at 0 hours withdrawal was 2.174 $\mu\text{g/g}$; and it was 1.920 at 24 hour withdrawal. At three days withdrawal the mean level was 0.958 $\mu\text{g/g}$. By seven days withdrawal, one pig had no detectable neomycin residue in kidney tissue, two pigs had $<0.5 \mu\text{g/g}$ and one pig had 0.991 $\mu\text{g/g}$. At 14 days withdrawal, three of the four pigs had no detectable neomycin residues in kidney tissue while one pig was assayed as having 0.906 $\mu\text{g/g}$ neomycin in its kidney tissue. Concentrations of neomycin in kidneys of swine are shown in Table 2 (Fagerberg, 1993).

Twenty-four (21 treated and 3 controls) swine of both sexes fed an average of 18.5 mg neomycin base/kg live weight/day for 14 days. Neomycin was not detected in liver, muscle, heart, or fat, at 0, 5, 8, 10, or 14 days withdrawal but was found to be 1.95 $\mu\text{g/g}$ (mean, $n=3$) in kidney at zero withdrawal and 0.84 $\mu\text{g/g}$ at eight days post treatment. The agar diffusion microbiological assay of Barbier et al. was used for analysis of tissues, although the limits of sensitivity were not mentioned (Barbiers, et. al., 1984).

Poultry

Residues of neomycin were studied by giving 10 mg or 30 mg/kg/day of neomycin to 4-week-old broilers for 7 days (detectable level: 0.05 μg (activity) per ml or g using a microbioassay method with *Staphylococcus epidermidis* as test organism). At 10 mg/kg/day, residues were detected in the kidney at day 10 after withdrawal. At 30 mg/kg/day, neomycin was detected in kidney and liver at days 13 and 3 after withdrawal, respectively (Ibayashi, 1994).

Broilers (150) were given feed containing 319 mg neomycin base per kg feed, which resulted in the animals receiving an average of 36.7 mg neomycin base/kg bird/day for seven days. Ten birds, five males and five females, were slaughtered on the last day of the treatment and on the five consecutive days following. Neomycin was not detected at any withdrawal time in liver or muscle at the sensitivity of the method which was 0.45 $\mu\text{g/g}$ for muscle and 0.75 $\mu\text{g/g}$ for liver (Strass, et. al., 1987a).

Hens (150) were fed 33.2 to 40.25 mg neomycin base/kg live weight/day. The animals were only dosed for five to seven days instead of twelve days as is recommended for residue studies in eggs. Eggs from 50 hens were sampled throughout the dosing period and at one day intervals for four days post treatment. Eggs from the other 100 birds were sampled before treatment and daily for 14 days withdrawal. Neomycin was not detected in any of the eggs at the detection limit of 0.45 µg/g. (Strauss, et. al, 1987b)

Turkeys

In this study, eight turkeys (4 of each sex) were gavaged with neomycin sulfate solution with a dose equivalent to 18.6 mg neomycin base/kg/day for 14 days. Two control birds of each sex were gavaged with sterile water for the duration of the study period. The animals were sacrificed at zero withdrawal, six hours past the last treatment, and livers from the controls and three each of the males and females were collected for assay. Neomycin was not detected in livers from the controls or in the female birds at the lower limit of quantification, 0.045 µg/g. In males, neomycin concentrations in liver were 0.16, 2.3, and 5.0 µg/g (George, 1988).

METHODS OF ANALYSIS FOR RESIDUES IN TISSUES

Neomycin has six cationic sites. The binding of neomycin to macromolecules is primarily ionic in nature. This binding can be disrupted by low pH and high concentrations of other cations. Analysis of neomycin in edible tissues requires extraction procedures that disrupt this binding to macromolecules.

Neomycin levels in all tissues can be determined by cylinder plate microbiological assay methods, using *Staphylococcus epidermidis*, with reported limits of detection between 0.15 and 1.25 µg/g. The specificity of the microbiological assay for neomycin is increased due to the high extraction temperatures and the length of the extraction. Many antibiotic, particularly the β-lactams are destroyed using the vigorous extraction conditions. However, other aminoglycosides may interfere with the analysis of neomycin (Barbiers, et. al., 1967) (Food and Drug Administration, USA, 1968) (Official Methods of Analysis, 1984).

Recent microbiological methods have released neomycin from macromolecules by extraction with 0.1N HCl plus 0.06M CaCl₂. Recovery from spiked tissue from four species has been reported to be 85-103% (Fagerberg, 1993; Ronning, 1993a, 1993b, 1994).

Neomycin in milk can be determined by a high performance liquid chromatograph (HPLC). Milk is passed directly through an Amberlite CG-50 ion exchange resin column, and the neomycin which is retained on the column is derivatized with ortho-phthalaldehyde. The derivatized neomycin is eluted from the column with potassium borate buffer/methanol and analyzed by HPLC. A HISEP HPLC column with fluorometric detection is used. The detection limit is 50 ng/g (Agarwal, 1990).

An HPLC method using an ion-pairing mobile phase, a reverse phase ODS column, post-column derivatization with o-phthalaldehyde reagent and fluorometric detection can measure residues of both neomycin B and C. The method is specific for neomycin and has a limit of detection of 0.10 µg/ml in plasma. The detector sensitivity was 3.5 ng of neomycin with a signal-to-noise ratio of 5 (Shaikh, et. al., 1985).

A screening method for the determination by HPLC in muscle of cattle, sheep and pigs and in muscle, liver, kidney and fat of veal calves uses a trichloroacetic acid/ethylene diamine tetraacetic acid method of extraction to break the ionic bonds. The limit of quantification for neomycin is 50 ng/g for muscle (Heitzman, R. J., 1994).

Changing the mobile phase of the above liquid chromatographic method eliminated variable retention times and frequent regeneration of the columns when analyzing for neomycin. The detection limit for kidney tissue was reported to be 0.5 µg/g. The method is specific because it distinguishes other aminoglycosides and antibiotics commonly used in food-producing animals. The method was used to determine neomycin in control samples of liver, muscle, milk, plasma and urine (Shaikh, 1993).

Residues of neomycin in bovine kidney can be determined and confirmed by HPLC/MS/MS. This method uses a matrix solid-phase dispersion method for tissue extraction. The method recovered 46% of the neomycin and had a limit of detection of 0.25 µg/g (McLaughlin, L. G., 1994).

APPRAISAL

Neomycin is poorly absorbed from the intestinal tract in man and animals (3% to 10%) and has low absorption from the udder. Absorbed neomycin accumulates in the kidney and, to a lesser extent, in the liver. It is not metabolized except by phosphorylation, adenylation, and acetylation in the digestive tract: absorbed neomycin remains as the parent compound. A ^{14}C -radiolabeled study in calves supports these conclusions except for very young animals (3-5 days of age). Orally administered neomycin is excreted primarily in the feces (>90%). Absorbed neomycin is excreted via the kidneys by glomerular filtration. After intramammary administration, neomycin is absorbed to a small extent in the lactating cow as evidenced by the neomycin found in the kidney for up to 14 days.

Numerous studies have been reported where neomycin was administered to farm animals at various dose levels and dosing times using primarily the oral route of administration. Those studies using the water soluble products are considered likely to result in the highest concentrations of residues in the edible tissues.

Tissue residue studies with neomycin have established parent neomycin as the marker residue and kidney to have the highest concentration of neomycin. The residues of neomycin in the kidney also persist the longest. This is true of all species tested. For all mammalian species, kidney was considered to be the target tissue. In poultry, kidney is considered inedible. Therefore, liver, the only other tissue with detectable residues of neomycin, is the target tissue for poultry.

At zero withdrawal periods, residue levels in the kidney of cattle, swine, chickens and turkeys were 2.8, 2.2, 8.4 and 5.0 mg/kg, respectively. At zero withdrawal the neomycin levels in milk (after oral administration) and eggs were both <0.2 mg/kg. The neomycin levels in kidney of sheep (one day withdrawal) and goats (12 hour withdrawal) were both 1.0 mg/kg. The kidney level in ducks at 14 days withdrawal was 0.89 mg/kg. The levels in milk from intramammary infusion of neomycin were 0.22 and 0.07 mg/l at 48 hours and 72 hours withdrawal, respectively. The residues in kidney after intramammary infusion at 14 days withdrawal were less than 5 mg/kg.

Adequate microbiological methods with a limits of quantification of 0.5 mg/kg in tissues and 0.2 mg/l in milk are available. HPLC and mass spectrometric methods are also available with limits of quantification of 0.1 mg/kg.

Maximum Residue Limits

Based on the residue studies and the temporary ADI of 0-30 $\mu\text{g}/\text{kg}$, temporary MRLs were established for parent neomycin in cattle, sheep, goats, pigs, chickens, turkeys and ducks:

Muscle	500 $\mu\text{g}/\text{kg}$
Liver	500 $\mu\text{g}/\text{kg}$
Kidney	5000 $\mu\text{g}/\text{kg}$
Fat	500 $\mu\text{g}/\text{kg}$
Eggs (Chicken)	500 $\mu\text{g}/\text{kg}$
Milk (Cattle)	500 $\mu\text{g}/\text{l}$

The ADI of 0-30 $\mu\text{g}/\text{kg}$ BW permits a daily intake of 1800 μg of neomycin for a 60-kg person. Using the above temporary MRL values, the theoretical maximum daily intake is muscle (300 g) 150 μg ; liver (100 g) 50 μg ; kidney (50 g) 250 μg ; fat (50 g) 25 μg ; eggs (100 g) 50 μg ; and milk (1.5 l) 750 μg . The total is 1275 μg per day.

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OXOLINIC ACID

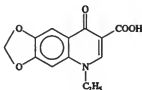
First draft prepared by
Dr. R. Wells
Australian Government Analytical Laboratories
Pymble, Australia

IDENTITY

Chemical names: 5-ethyl-5,8-dihydro-8-oxo-1,3-dioxolo[4,5-g]quinoline-7-carboxylic acid;
1-ethyl-1,4-dihydro-6,7-methylenedioxy-4-quinolone-3-carboxylic acid

Synonyms: W 4565, Emyrenil, Nidantin, Ossian, Ozoboi, Pietil, Prodoxol, Urinox,
Uritrate, Uro-Alvar, Urotrate, Uroxin Von Boeb, Uroxol, Utibid.

Structural formula:



Molecular formula: $C_{13}H_{11}NO_5$

Molecular weight: 261.24

OTHER INFORMATION ON IDENTITY AND PROPERTIES

Pure active ingredient: Oxolinic acid

Appearance: Colourless powder or crystals

Melting point: 314-316°C

Optical rotation: Optically inactive

Mode of Action: Detailed studies were made of the effect of oxolinic acid on the growth of *Proteus vulgaris*. Using appropriate culture media, the organism was grown in the presence of a number of isotopically labelled precursors of cell constituents. These included thymidine, uracil and L-valine incorporation (Parke-Davis, 1994).

It was found that exposure of growing cells to various concentrations of oxolinic acid markedly inhibited DNA synthesis in a dose-related manner. Protein synthesis was also suppressed, but to a lesser extent. Oxolinic acid had no effect on the synthesis of RNA. Furthermore, the cell wall was not altered by oxolinic acid as shown by no leakage of d-glucose and no depression of cell wall lipid.

Indications: The major use of the substance is in aquaculture, both as a therapeutic and prophylactic agent in fish.

Oxolinic acid is a synthetic antibacterial agent used in many countries in the treatment of fish diseases. The compound has been used successfully for the control of furunculosis (Endo et al., 1973; Austin et al., 1983), vibriosis

(Endo et al., 1973; Austin et al., 1982) and enteric redmouth disease (Rodgers and Austin, 1983). Oxolinic acid is administered to fish mixed in the feed usually for 10 days at doses of 10-30 mg/kg body weight.

Fish: Furunculosis, yersiniosis, vibriosis, cold-water vibriosis

RESIDUES IN FOOD AND THEIR EVALUATION

CONDITIONS OF USE

General

Oxolinic acid is a synthetic antimicrobial agent which is similar in structure to the naturally occurring nalidixic acid, but is more active against both Gram-negative and Gram-positive bacteria although it is used almost exclusively for its Gram-negative microbiological activity. Oxolinic acid is an older member of a group of synthetic antimicrobial agents generically termed the *quinolones*. Although more recent additions to the quinolone drug family out-perform oxolinic acid with respect to both bacteriocidal activity and bioavailability, its relatively modest cost, low mammalian and fish toxicity and satisfactory performance render it a useful and widely used drug, particularly in the aquaculture industry. Human oral absorption of oxolinic acid is low. It is excreted in the urine and has been used in human medicine for the treatment of urinary tract infections, although alternative treatments are often preferred.

Oxolinic acid has shown activity against a broad range of Gram-negative organisms, being especially active against proteus species but less active, or inactive, against pseudomonas.

The spectrum of antimicrobial activity of oxolinic acid is similar to that of nalidixic acid except that oxolinic acid is also active against staphylococci. Oxolinic acid is not particularly effective against other pathogenic Gram-positive bacteria.

Oxolinic acid is also used in veterinary medicine for the treatment of disorders arising from Gram-negative infections. However, by the far the major use of the drug is in the aquaculture industry where, in addition to its Gram-negative action, its broad spectrum of activity against fungi, protozoans and helminths have resulted in its effective use in fish farming in many countries, both as a prophylactic as well as a chemotherapeutic agent.

Oxolinic acid has a high activity against *Aeromonas salmonicida*. The Minimum Inhibitory Concentration (MIC) values ranged between 0.0075 - 0.03 µg/mL for 10 isolates tested. These MIC values were markedly lower than the corresponding MIC values for nalidixic acid which ranged between 0.06 - 0.125 µg/mL (Parke-Davis, 1994).

Further experiments again showed the sensitivity of pathogenic strains of *A. salmonicida* to oxolinic acid and also showed the quick inhibiting effect on bacterial cultures of *A. salmonicida* insofar as the bacterial cultures were inhibited within 5 minutes by a drug concentration of 50 mg/L. In field studies on salmon, trout and carp, oxolinic acid was found to be effective in treating *A. salmonicida* infections at doses between 5-20 mg/kg, whereas nalidixic acid was much less effective.

Endo et. al. (1984) also showed that oxolinic acid had a MIC of 0.02 µg/mL against the one strain of *A. salmonicida* tested and also demonstrated the MIC of oxolinic acid against the 7 strains of *A. liquefaciens* tested ranged between 0.02-0.09 µg/mL and against the 5 strains of *Vibrio anguillarum* tested with a range of 0.02 to 0.09 µg/mL.

Dosage

Species:	fish species
Routes of administration:	oral
Dose	10-50 mg/kg

PHARMACOKINETICS

General

Very little if anything, is known about the distribution or the degree of protein binding of oxolinic acid in different tissues and organs (Hustvedt and Salte, 1991). Similarly, there is no information as to the pattern of distribution of oxolinic acid in fish, i.e. whether the drug is homogeneously distributed.

In rainbow trout, there was a significantly lower terminal elimination half-life in seawater than in freshwater. This difference was attributed to the higher total clearance in seawater, i.e. the sum of all elimination processes in the fish. This may be caused by differences in the rate of elimination, metabolism or both. The urine flow rate in salmonids is dramatically reduced in seawater compared to freshwater (Hunn, 1982). Thus, renal excretion of the drug could not be a rate-limiting step in the elimination of oxolinic acid under usual experimental conditions. Approximately 50% of oxolinic acid in the plasma at therapeutic concentrations is unbound (Benjaminsen and Hustvedt, 1986) and can freely interact with the secretory system in the nephrones. The secretion of some xenobiotics is higher in fish compared with mammals (Pritchard and Bend, 1984), this may also be true for oxolinic acid.

Giant Prawn (*Penaeus monodon*)

The pharmacokinetics of oxolinic acid haemolymph clearance, absorption, tissue distribution and excretion were investigated in the giant prawn (*Penaeus monodon*) after single intramuscular injection and oral administration to prawns of 30-40 g body weight at 28-32°C (Limpoka et al., 1994). Elimination half-life was 4.68 hours following intramuscular injection of 10 mg/kg of oxolinic acid with an apparent volume of distribution of 0.22 L/kg. At a higher dose of 20 mg/kg the distribution phase revealed a half-life of 3.36 h and an elimination half-life of 5.37 hours. No drug levels occurred in tissue above 10 ng/g 5 days after injection. The drug was rapidly absorbed after single oral dosing. The peak haemolymph concentration of 0.45 µg/mL and 0.55 µg/mL occurred 0.87 and 0.81 hours after forced oral administration, respectively. There was a good correlation between the concentration of the drug found in haemolymph and tissue levels. No drug levels occurred in tissue above 10 ng/g 3 days after the single oral dose.

Prawns were fed for 5 days on a diet containing oxolinic acid at 0.5 g/kg and 1.0 g/kg in the feed respectively. The edible tissues of prawns contained less than 10 ng/g oxolinic acid six days after withdrawal of medicated pelleted feed and 7 days after withdrawal of medicated fish flesh diet. The haemolymph and tissue concentrations of oxolinic acid during the feeding of prawns with medicated fish flesh feed for 5 days followed by withdrawal from the drug is shown in Table 1.

Giant Sea Perch (*Lates calcarifer*)

The clearance of oxolinic acid from plasma and tissue in the Giant Sea Perch (*Lates calcarifer*) after single oral administration and 5 days on medicated diet has been investigated (Chai-anan et al., 1993). Following a single oral dose of 20 mg/kg, peak plasma and tissue concentrations were found after 1 hour and fell below the limit of detection in 72 hours post-dosing. There was a good correlation between the concentration of the drug found in plasma with levels in edible tissues at each sampling time point (Table 2).

Fish were fed for 5 days on a diet containing oxolinic acid at 1.0 g/kg feed. The plasma contained above 1 µg/g oxolinic acid during medicated feeding. Plasma levels of oxolinic acid fell below the detection limit of 200 ng/kg on day 8 after withdrawal of medicated diet. There was a good correlation between the concentration of the drug found in plasma with levels in edible tissues, the ratio at each sampling time point being 1.1-1.5 : 1. Therefore, for fish maintained at 30°C and fed on a medicated diet of 1 g/kg of oxolinic acid, a drug withdrawal period of 8 days was recommended.

Table 1. Mean Prawn Tissue and Haemolymph Concentrations of Oxolinic Acid Following Adding 1 g and 2 g Aquinox/kg Fish Flesh Feed for 5 Days¹.

Time (hr)	Days	Haemolymph Conc. ($\mu\text{g}/\text{mL}$) ²		Tissue Conc. ($\mu\text{g}/\text{g}$)	
		Dose 1 g/kg	Dose 2 g/kg	Dose 1 g/kg	Dose 2 g/kg
0	1	0	0	0	0
4		0.82 ± 0.12^3	1.15 ± 0.18^3	1.83^3	2.89^3
24	2	0.65 ± 0.30	1.00 ± 0.16	1.25	1.70
28		0.75 ± 0.10	1.28 ± 0.10	1.30	5.11
48	3	0.50 ± 0.30	0.75 ± 0.10	1.20	1.80
52		0.85 ± 0.20	0.92 ± 0.07	1.30	2.89
72	4	0.58 ± 0.20	0.97 ± 0.27	0.98	1.82
76		0.93 ± 0.20	1.20 ± 0.50	0.44	3.34
96	5 ⁴	0.80 ± 0.05	1.0 ± 0.20	1.10	1.67
98		1.0	1.37 ± 0.30	4.40	5.09
100		0.09 ± 0.07	1.20 ± 0.16	1.70	3.22
104		0.87 ± 0.08	1.0	1.60	1.60
120	6	0.60	0.93 ± 0.90	0.70	1.94
144	7	0.25	0.825 ± 0.17	0.50	0.85
168	8	trace	0.25	0.45	0.50
192	9	ND	0.25	0.35	0.40
216	10	ND	trace	0.02	0.20
248	11	ND	trace	ND	0.10
264	12	ND	ND	ND	ND

1. Pharmsure Aquinox = 50% oxolinic acid

2. Mean from four different haemolymph samples, microbiological assay, LOD 250 ng/g.

3. Assay by HPLC, limit of detection = 10 ng/g, mean from pooled samples.

4. The last day of drug dosing

ND = not detected; trace = < 250 ng/g

Table 2. Mean Fish Tissue and Plasma Concentrations of Oxolinic Acid Following a Single Oral Dose of 20 mg/kg

Time (hours)	Oxolinic acid concentration		Ratio of concentrations Plasma/Tissue
	($\mu\text{g}/\text{mL}$)	($\mu\text{g}/\text{g}$)	
	Plasma ¹	Tissue ¹	
0.3	0.85 ± 0.40	0.80	1.06
1	1.25 ± 0.15	1.05	1.19
2	0.50 ± 0.10	0.40 ± 0.20	1.25
4	0.45 ± 0.20	0.30 ± 0.01	1.50
8	0.25	0.02 ± 0.015	1.25
12	<0.25	0.40 ± 0.01	
24	<0.25	0.30	
30	<0.25	0.02	
48	trace	0.01	
72	n.d.	n.d.	

¹ Mean from three individual fish; n.d. = not detected

Atlantic salmon (*Salmo salar* L.)

Hustvedt (1993a) has reported on the administration of oxolinic acid as a single dose to Atlantic salmon (*Salmo salar* L.). Twelve fish weighing 923 ± 202 g, maintained between 8.0 and 8.1°C, were given a mean single

oral dose of 25.9 mg oxolinic acid per kg body weight administered as 0.5 % medicated pellets. Blood samples were taken via a cannula at 3, 6, 9, 12, 24, 48, 96, 144 and 192 hours after the administration of oxolinic acid. The serum samples were cleaned up by a solid phase extraction procedure and oxolinic acid concentrations were determined in duplicate by high-performance liquid chromatography as described by Thanh (1988). The analytical method gave recoveries of 82 %, 85 % and 90 %, and the coefficients of variation were 8 %, 6 % and 5 % at 0.01, 1.0 and 5.0 $\mu\text{g/mL}$, respectively. The detection limit (signal to noise ratio 3:1) was 1 ng/mL.

The bioavailability of oxolinic acid in this study was estimated by using the pharmacokinetic constants following a single intravascular injection of oxolinic acid presented by Hustvedt et al. (1990b). Thus AUC was estimated to be 782 g·b·mL⁻¹ after 20 mg oxolinic acid per kg body weight. The elimination processes were all assumed to follow first order kinetics. All pharmacokinetic values were determined according to Rowland and Tozer (1989). The mean values were estimated based upon mean serum concentrations from all fish (n=9).

The estimated mean bioavailability of oxolinic acid after a single oral dose of 0.5 % medicated feed was 19.9 %. The mean fraction of available dose remaining in the fish at 192 h was 0.075. Mean T_{max} was 24.3 b (range 17.0-33.3 b), and C_{max} was 2.1 $\mu\text{g/mL}$ (range 1.5-4.0 $\mu\text{g/mL}$) while the lag time (T_{lag}) was 2.0 b (range 0-4.2 b). Over all mean elimination half-life was 55.4 b while individual fish ranged from 28.4 to 65.2 b. Although the bioavailability of oxolinic acid was low, serum levels which were achieved should be sufficient to obtain satisfactory serum levels after multiple dosing.

The serum profile of oxolinic acid after multiple doses has been studied by Hustvedt (1993b). Fish, maintained at a mean water temperature of 7.8°C and a mean salinity of 26‰ NaCl, were fed medicated feed containing 0.5 % oxolinic acid to a feeding level of 0.5 % of body weight divided into 4 times/day for 2 consecutive days followed by medicated feed every second day for 6 days (total of 6 days with medicated feed). The fish were starved between medicated feeding. Blood samples were taken at 3, 8, 24, 27, 32, 48, 51, 56 and 72, 96, 120, 144, 168, 192, 216 and 240 h after the commencement of the treatment.

An average serum concentration of 4 $\mu\text{g/mL}$ was achieved between 2 and 3 days after the commencement of the treatment. During the treatment period, the lowest determined serum concentration of oxolinic acid (C_{min}) was 1.6 $\mu\text{g/mL}$ (sample No. 149 taken at 216 h). The highest determined serum concentration of oxolinic acid (C_{max}) was 8.6 $\mu\text{g/mL}$.

The plasma profile after the oral multiple dosage regimen corresponded well with an estimated profile based solely upon the pharmacokinetics of the single dose study presented above, i.e. C_{max} 4.1 $\mu\text{g/mL}$ after 48 h and 3.9 $\mu\text{g/mL}$ after 218 h. The dosage regimen used should be sufficient to maintain the serum levels of oxolinic acid between 3 - 5 $\mu\text{g/mL}$ in fish sera.

Estimation of withdrawal time of oxolinic acid has also been reported by Hustvedt (1993c). A statistical approach to the setting of withdrawal time was used. It was suggested that the time intercept between detection limit of the analytical method and the upper 90 % prediction limit of a linear regression on the logarithm of the serum concentration provided a realistic estimate of necessary withdrawal time as 35 days. Tissue samples were analyzed to test the suggested withdrawal time. Concentrations of oxolinic acid were followed in a group of fish post treatment. Blood samples from 10 fish were taken at 2, 6, 14, 21, 28, 35, 42 and 49 days after the end of the treatment. Muscle, liver and kidney samples from 8 of these fish were taken at 14, 21, 28, and 35 days post treatment.

At a mean temperature of 8.2°C, the level of oxolinic acid was predicted to fall below 1 ng/mL with a probability of 95 % at 35 days post-treatment. The serum concentration of oxolinic acid was shown to fall below the detection limit of the analytical method within 28 days post treatment, corresponding values in muscle, liver and kidney were between 28 and 35 days. Tissue samples fell below the detection limit of the analytical method within the suggested 35 days. In contrast, Michel (1986) recommended a withdrawal time of only 6 days. Archimbault et al. (1988) also proposed the withdrawal time to be 6 days on the base of a tolerance level of 0.05 ppm following a dosage-level of 12 mg per kg per day for 7 consecutive days at 9-10°C. Jacobsen (1989) suggested a withdrawal time of 20 days after dosing at 10 mg per kg per day for 10 days in rainbow trout kept in freshwater at approximately 18°C (*vide infra*).

It has been demonstrated (Ishida, 1990b) that the change in oxolinic acid levels in seawater-acclimatised coho salmon after oral administration was different from that in freshwater coho salmon. It was also found that the change in oxolinic acid levels in seawater coho salmon were similar to those in seawater fishes such as Japanese mackerel, red sea bream, yellowtail, and flounder. Oxolinic acid therefore appears to be retained at higher concentrations and for longer times in the freshwater fishes than in the seawater fish. Experiments with rainbow

trout (*vide infra*) supports this conclusion. It is suggested that the difference of the change in oxolinic acid levels after oral administration must be a function of the salinity of the fish's environment (Ishida, 1992).

Hustvelt et. al. have studied the therapeutic use of oxolinic acid in combatting the infection of Atlantic salmon infected with *Vibrio salmonicida*, the causative agent of cold-water vibriosis. Mean serum levels were maintained above 1.2 µg/mL for more than 10 days by oral treatments of 15 mg/kg body weight of oxolinic acid per day. A total dose of 75 mg oxolinic acid per kg fish was given at seawater temperatures between 2°C and 4°C (site 1), while a total dose of 90 mg per kg fish was given at seawater temperatures between 13°C and 15°C (site 2). Mortalities ceased between 3 and 4 days after initiation of treatment in both cases. Based on measured serum concentrations of oxolinic acid post-treatment, withdrawal periods of 38 days at 4-6°C and 31 days at 13-15°C were necessary to ensure (with a probability of 95%) that the drug levels would remain below 1 ng per mL serum.

In a comparison of the efficacy of oxolinic acid with that of nalidixic acid against *A. salmonicida* infections (furunculosis) Atlantic salmon were force fed with between 5 and 20 mg/kg of each drug (Parke-Davis, 1994). The first dose of medicated feed was followed by a second dose after 4 days. At all dose levels, oxolinic acid treated fish survived whereas there were mortalities in nalidixic acid treated fish, even at the highest dose. The oral administration of 10 mg oxolinic acid per kg fish to freshwater and seawater Atlantic salmon produced serum oxolinic acid levels which, in all cases, exceeded by many times the highest 48 hour MIC value of 0.03 mg/mL observed for *A. salmonicida* strains.

The serum level obtained in freshwater salmon following 7 daily consecutive feeds at 10 mg oxolinic acid per kg are in the same range as those obtained in seawater salmon as well as those of freshwater carp (Endo et. al., 1973). There is a marked variation in serum levels with individual fish but this can be expected due to a variable feeding response (Parke-Davis, 1994, p. 69).

Serum levels in freshwater salmon are higher in the samples taken after 10 consecutive daily feeds than those taken after the 7 consecutive daily feeds. This may be due to some accumulation of the drug although this was not evident in seawater, or it may be a reflection of a variable feeding response. The second possibility is supported by the wide range of values present. The test population of freshwater salmon parr in this experiment was much smaller than the normal population occupying a tank of the size used and this may well have affected the feeding response of the fish.

Oxolinic acid does not persist in edible salmon tissues beyond 7 days following a 10 day feeding period at 10 mg/kg, although a trace of oxolinic acid was recorded in the kidney tissue at 7 days post final treatment. Rapid elimination of oxolinic acid was also shown by Endo et. al. (1973) following oral administration to carp. From these results, a withholding period of 14 days is recommended between final treatment and culling of fish for human consumption.

An increase in the dose of oxolinic acid above 10 mg/kg leads to an increase in serum levels, but there is a levelling of serum concentration above a dose of 80-160 mg oxolinic acid per kg fish. The absence of mortality in fish receiving this dosage demonstrates that the serum levels attained are not toxic to the fish. The levelling off of the serum concentration also suggests that drug absorption may be restricted at oral doses in excess of 160 mg/kg.

From mammalian studies it was found that oxolinic acid is metabolised into a number of pharmacologically active metabolites. If it is assumed that in fish metabolism of oxolinic acid is similar, it would be impractical to chemically assay the sum of active metabolites in fish. Hence a microbiological assay method (Finlay and Stevenson, 1994) was chosen as a reliable method of indication of oxolinic acid level in the tissue, rather than other methods assaying pure oxolinic acid (Parke-Davis, 1994, p. 69).

Serum level data for oxolinic acid in Atlantic salmon found in this work is detailed in Tables 3 - 6.

Table 3. Mean Serum Levels of Oxolinic Acid for Atlantic Salmon in Seawater Determined from Samples Taken at Various Time Intervals Following 6 Consecutive Daily Dietary Doses (Alderson and Finlay, 1994).

Oxolinic Acid Dose Rate (mg/kg)	Mean Serum Levels ($\mu\text{g/mL}$)			
	6h	24h	48h	72h
5	0.76	-	0.07	0
10	1.61	0.81	0.33	0.15
20	2.61	-	0.55	0.37

Table 4. Mean Serum Levels of Oxolinic Acid for Atlantic Salmon in Seawater Determined from Samples Taken at Various Time Intervals Following 3 Daily Dietary Doses (Alderson and Finlay, 1994).

Oxolinic acid Dose Rate (mg/kg)	Mean Serum Levels ($\mu\text{g/mL}$)		
	6h	24h	96h
40	2.8	3.6	n.d.
80	5.3	4.6	n.d.
160	7.2	5.2	n.d.
320	5.0	5.4	trace
640	6.1	4.8	n.d.

n.d. = not detected

Table 5. Mean Serum and Tissue Levels of Oxolinic Acid for Seawater Atlantic Salmon after 10 Consecutive Daily Dietary Doses of 10 $\mu\text{g/kg}$ (Finlay and Alderson, 1994).

	Mean Oxolinic Acid Level ($\mu\text{g/mL}$)		
	6h	24h	72h
Serum	1.64	0.98	0.27
Liver	1.12	0.19	trace
Kidney	1.12	0.20	trace
Muscle	1.79	0.29	trace

Table 6. Mean Serum and Tissue Levels of Oxolinic Acid for a Caged Population of Seawater Atlantic Salmon after 10 Consecutive Daily Dietary Doses of 10 $\mu\text{g/kg}$ (n=5) (Rae et. al., 1994).

Day after last dose	Mean Oxolinic Acid Level ($\mu\text{g/mL}$)		
	Day 1	Day 4	Day 7
Serum	1.39	trace	n.d.
Liver	0.40	trace	n.d.
Kidney	0.29	0.04	trace

n.d. = not detected

Brown Trout (*Salmo trutta*)

Rainbow Trout (*Oncorhynchus mykiss*)

In a study on rainbow trout kept in freshwater and in seawater at 8.5°C, elimination half-life, apparent volume of distribution and total clearance of oxolinic acid were estimated after a single intravascular injection. The drug was given as a rapid injection through a cannula into the dorsal aorta. Repeated blood samples were taken via the cannula and serum was analysed by reversed-phase high-performance liquid chromatography (Hustvedt and Salte, 1991).

The terminal elimination half-life for oxolinic acid in rainbow trout, estimated to be 52.6 h in freshwater ($t_{1/2}$), was significantly lower than that in sea water (29.1 h). This considerable difference in elimination half-lives was mainly due to the increase in total clearance. Total clearance values were 1.2 and 2.0 L·kg⁻¹·24h⁻¹. The apparent volume of distribution was estimated to be 2.9 and 2.61 L/kg in freshwater and seawater, respectively. The large apparent volume of the central compartment in rainbow trout kept in freshwater indicated that oxolinic acid was initially rapidly distributed to tissues outside of the blood volume of the fish. Furthermore, the large apparent volume of distribution indicated that more than 90% of the drug in the fish was outside the serum.

The concentrations of oxolinic acid in tissues of seawater-acclimatised rainbow trout were compared with those of fresh water rainbow trout after a single oral administration (40 mg/kg). Up to 24 h after administration, little difference in the tissue concentrations was observed between the two groups of trout. However, tissue concentrations of the drugs in seawater trout decreased to undetectable levels by 72 h, whereas the concentrations in the freshwater trout peaked at 48 h and were detectable for at least 244 h. The kinetics of oxolinic acid uptake and excretion in the seawater trout were similar to those reported for seawater teleosts. Both groups of trout metabolized oxolinic acid by the same pathway, because the same oxolinic acid metabolites were observed in the bile of both groups of trout after a single oral administration. When oxolinic acid was injected (20 mg/kg) into the caudal vessels of each group of trout, serum levels of oxolinic acid decreased immediately after injection in both groups. Serum concentrations were 8.69 ± 2.14 µg/mL in the freshwater trout and 8.03 ± 2.27 µg/mL in seawater trout by 3 h after injection, respectively. Subsequently, the serum levels of oxolinic acid in the seawater trout decreased to barely detectable levels by 72 h. In contrast, oxolinic acid levels in freshwater trout were higher and persisted for longer, up to 244 h. These results confirm that oxolinic acid is excreted more rapidly in seawater trout than in freshwater trout.

Endo et al. (1987) had previously determined that there was a difference in the elimination of oxolinic acid between different fish species by comparing their data for yellowtail with data reported for rainbow trout and ayu by Kasuga et al. (1984).

The pharmacokinetics and bioavailability of oxolinic acid were studied in rainbow trout at a water temperature of 16°C after intravascular and oral dosing (Bjorklund and Bylund, 1991). 10 Fish weighing 514 ± 14 g were given an intravascular dose of 10 mg/kg oxolinic acid and 5 fish weighing 501 ± 20 g were dosed orally at 75 mg/kg in this work.

The pharmacokinetics were best described by a two-compartment open model giving distribution half-lives of 0.31 h and 1.53 h, and an elimination half-life of 69.7 h for oxolinic acid with a volume of distribution of 1.94 L/kg. The elimination half-life, total clearance time and apparent volume of distribution was generally in line with the work of Hustvedt and Salte (1991). The apparent oral bioavailability for oxolinic acid was 13.6% and the plasma protein binding was 27%. The drug was well tolerated with an acute oral toxicity (LD₅₀) exceeding 4000 mg/kg.

The temperature-related absorption and excretion of oxolinic acid in rainbow trout has been studied by Bjorklund et al. (1992). Absorption and elimination in serum, bile and tissues were studied at 5, 10 and 16°C in fresh water fish weighing 266 ± 48 g after a single oral dose of 75 mg/kg oxolinic acid. The maximum levels of oxolinic acid were obtained in serum within 1, 4 and 6 days at 16, 10 and 5°C, respectively. The highest drug concentrations were measured in bile followed by liver, kidney, muscle tissue and serum. The higher levels of oxolinic acid in tissues compared to serum indicate a good distribution of the drug. The elimination half-life in serum was 24 h at 16°C, 4.0 days at 10°C and 6.1 days at 5°C. With a detection limit of 0.01 µg/g for the oxolinic acid in the HPLC assay, the predicted withdrawal time with 95% confidence for muscle tissue was 28 days at 16°C, 60 days at 10°C and 140 days at 5°C. Results obtained under laboratory conditions were similar to those from field trials. These results are summarised in Table 7.

Table 7. Elimination Rate Constants (β), Half-lives ($t_{1/2}$) and Predicted Withdrawal Times (t_{pred}) for Oxolinic Acid in Tissues of Rainbow Trout at Different Temperatures.

Tissue	Temp (°C)	$\beta \pm \text{SD/day}$	$t_{1/2} \pm \text{SD (days)}$	$t_{pred} \text{ (days)}^*$
Serum	5	0.114 $\pm$ 0.010	6.1 $\pm$ 0.8	125
	10	0.171 $\pm$ 0.028	4.0 $\pm$ 1.0	54
	16	0.711 $\pm$ 0.033	1.0 $\pm$ 0.06	15
Muscle	5	0.103 $\pm$ 0.016	6.7 $\pm$ 1.5	140
	10	0.151 $\pm$ 0.007	4.6 $\pm$ 0.3	60
	16	0.354 $\pm$ 0.037	2.0 $\pm$ 0.3	28
Liver	5	0.091 $\pm$ 0.013	7.6 $\pm$ 1.6	270
	10	0.102 $\pm$ 0.010	6.8 $\pm$ 1.0	165
	16	0.186 $\pm$ 0.015	3.7 $\pm$ 0.4	60
Kidney**	16	0.192 $\pm$ 0.010	3.6 $\pm$ 0.3	44

Fish were given a single oral dose of 75 mg/kg oxolinic acid/kg body weight. Each value is a mean of five fish.

* The predicted withdrawal times (with 95% confidence) are based on a detection limit of 0.01 $\mu\text{g/g}$ for the HPLC assay.

** Levels of oxolinic acid in kidney were not measured at 5 and 10°C.

Oxolinic acid, oxytetracycline and trimethoprim were investigated in whole gutted rainbow trout together with their skin, muscle and blood, at 6, 12 and 18°C. Concentration of drugs were measured by HPLC. It is recommended that withdrawal times for rainbow trout be based on whole gutted rainbow trout and not, as in earlier investigations, from residual concentrations in muscle. Oxolinic acid in rainbow trout feed was found to be stable during the pelleting process used and up to 81 days after pelleting. The absorption of the drug in rainbow trout shows different temperature dependencies. A quantitative comparison data from this work with individual results from earlier investigations suggested withdrawal times for freshwater rainbow trout of about 20 days at approximately 18°C.

Archimbault et. al. (1988) studied the serum and tissue concentrations of oxolinic acid in rainbow trout (approximate weight 200 g) fed orally at a rate corresponding to 12 mg/kg/day for 7 consecutive days. This dosing allowed a maintenance of tissue and serum levels of oxolinic acid in excess of appropriate MICs for two fish pathogens. At the end of the treatment period, tissue concentrations of 1.97 $\mu\text{g/g}$ were found which had declined to 0.05 $\mu\text{g/g}$ at 6 days and 0.03 $\mu\text{g/g}$ at 10 days after withdrawal of the medicated feed in fish maintained at 9–10°C.

The prophylactic efficacy of oxolinic acid against *A. salmonicida*-infection of rainbow trout has been investigated (Parke-Davis, 1994). Two groups of fish, an 0+ group (100 x 3.5–5 g) and a 1+ group (30 x 600–650 g), maintained in aerated freshwater at 18°C were administered oxolinic acid at doses of 10 mg/kg for 10 days. All of the fish survived at all dosing levels whereas 56% of 0+ and 50% of 1+ control fish had died 9 days after challenge.

The efficacy of oxolinic acid in the protection of brown trout challenged with *A. salmonicida* infections has been established (Parke-Davis, 1994). Fish, maintained in aerated freshwater at 18°C, were administered oxolinic acid incorporated into the food for 10 days, commencing at 4 days post-challenge with *A. salmonicida*. At doses of 75 and 50 mg/kg, all of a group of 50 fish survived and, at 5 and 25 mg/kg, only one of a group of 50 fish died. By contrast all fish in the untreated control group died.

In a parallel study (Parke-Davis, 1994), the prophylactic efficacy of oxolinic acid against *A. salmonicida* infection of brown trout was investigated. Groups of 50 fish, maintained in aerated freshwater at 18°C, were administered oxolinic acid at doses of 5, 25, 50 and 75 mg/kg. All of the fish survived at all dosing levels whereas all of the 50 control fish had died 10 days after challenge.

Eels

The *in vitro* antibacterial activity of oxolinic acid on diseased eels has been studied (Liu, 1991). Pharmacokinetics of oxolinic acid administered to eels was studied together with its efficacy against artificial infections of *Aeromonas hydrophila* and *E. tarda*.

Results obtained from an *in vitro* antibacterial study showed that minimal inhibitory concentrations of oxolinic acid against *Aeromonas hydrophila*, *E. tarda* and *Pseudomonas anguilliseptica* were 0.10-0.78, 0.10-0.39, and 0.10-0.20 µg/mL, respectively; whereas those against *Flexibacter columnaris* were 6.25-25.1 µg/mL. In an efficacy trial, eels were artificially infected with *A. hydrophila* and *E. tarda*, respectively, and oxolinic acid was administered on each 3rd consecutive day with a single dose of 8, 12 and 16 mg/kg body weight per day, respectively. The results showed that a significant difference ($p < 0.05$) of the cumulative mortalities was obtained between the control group and the groups treated with 12 mg/kg b.w. or those with 16 mg/kg bw ($p < 0.05$). No pathogens were isolated from the survivors for each medicated group. However, tested bacteria could be isolated from the survivors of the control eels.

In a pharmacokinetic study, 3 time course patterns for various concentrations of oxolinic acid in serum and tissues were found. In the first pattern, the highest concentration in serum, liver and muscle occurred 3 hours post-administration. The concentration of drug in serum and tissue then declined and the second concentration peak was detected at 12 hours after oral administration. Subsequently the drug concentration decreased with time. In the second pattern, the maximum concentration in gill was obtained at 6 hours post-administration and then declined rapidly with time. In the third pattern, highest drug concentration in kidney was found at 6 hours post-administration followed by a slow decrease. The second concentration peak in kidney was detected at 24 hours after oral administration which decreased gradually with time.

Twelve days after administration of oxolinic acid at a dosage level of 12 mg/kg bw for 3 consecutive days, no drug residue was detected, either in eel serum or tissue.

Chickens

Archimbault and Ambroggi (1987) investigated the use of oxolinic acid in poultry. A serum bioavailability study was performed after oral administration in hens of a single dose of 10 mg/kg and after repeated doses of 15 mg/kg/day for 5 consecutive days. Lung concentration and residue levels in eggs were also evaluated after administration of repeated doses using an analytical method with a detection limit of 10 ng/g or mL. Overall studies confirmed the therapeutic potency in poultry of a dose of 15 mg/day and gave an estimation of the oxolinic acid residues in tissues and eggs. If a maximum residue limit of 50 µg/kg is adopted, the elimination of oxolinic acid requires a withdrawal period of 6 days for tissues and 8 days for eggs.

Anadon et. al. (1990) studied the pharmacokinetics and residue depletion of a series of quinolone compounds and of olaquinox in poultry. Oxolinic acid (15 mg/kg) fed orally to 8 x 40 day old broiler chickens (Hubbard x Hubbard, weighing 2.5 kg) gave the following mean pharmacokinetic parameters: T_{max} (h) 2.7 ± 0.14 , C_{max} (µg/mL) 11.93 ± 0.29 , $t_{1/2}$ (h) 33.54 ± 9.88 , and AUC (mg·h/L) 478.10 ± 33.10 .

Mean tissue concentrations of oxolinic acid following oral administration of 200 mg/kg to 6 x 40 day old broiler chickens (Hubbard x Hubbard, weighing 2.5 kg) are shown in Table 8.

Table 8. Mean Tissue Concentrations of Oxolinic Acid Following Oral Administration of 200 mg/kg to 6 x 40 Day Old Broiler Chickens (Hubbard x Hubbard, Weighing 2.5 kg)

Tissue	Oxolinic Acid Concentration (µg/g)			
	Day 1	Day 3	Day 6	Day 8
Muscle	1.46 ± 0.16	0.57 ± 0.15	0.02 ± 0.01	n.d.
Liver	2.16 ± 0.13	0.49 ± 0.06	0.05 ± 0.01	n.d.
Kidney	2.38 ± 0.35	0.91 ± 0.45	0.16 ± 0.05	0.05 ± 0.01

n.d. = not detected. Quantification by HPLC (Horie et. al., 1987)

Based on these results, the authors suggest 8 days as an appropriate withholding period for poultry after cessation of medicated feed.

METABOLISM

Introduction

Oxolinic acid has been a widely used prophylactic and chemotherapeutic agent in aquaculture over a decade and the pharmacokinetics of the substance have been studied in fish and animals as well as man. The few metabolic studies of oxolinic acid which have been carried out were relatively early works and the structure of many of the metabolic products were not elucidated.

Although the pharmacokinetic behaviour of oxolinic acid has been well studied in many fish species, the metabolism of the drug in these species has not been studied in any depth and the fate of the drug is largely a matter of conjecture. In the absence of any definitive metabolism study in fish, the known metabolism in other species are discussed below. This does not necessarily indicate that the metabolism in fish is similar.

Metabolism in Man

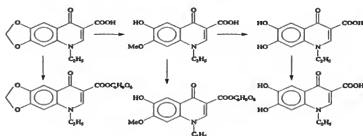
Human metabolism of oxolinic acid is considered here because of a general dearth of information about its metabolism in those animal and fish species where it is most widely used. In man (4 subjects treated with a single 1 g oral dose of drug), oxolinic acid is converted into at least 8 metabolites which are excreted principally as glucuronides (Di Carlo et. al., 1968 a,b). The peak blood radioactivity was observed 4 hours post-dosing when 1.2% of the radioactivity was in the systemic circulation. The elimination of radioactivity into the urine and faeces averaged 42.7% for the first 24 hours and 66.7% over the whole study period of 48 hours. Both urine and faeces demonstrated antimicrobial activity although free oxolinic acid was not present. The radioactivity in the zero to 6 hour collections was found to consist of 43.4% oxolinic glucuronide, 1.4% labile complex of oxolinic acid, 37.5% glucuronides derived from modified oxolinic acid and 17.7% non-glucuronide metabolites.

The glucuronides of both parent drug and some metabolites are unstable in aqueous solution. Thus in the case of oxolinic acid glucuronide, the active parent drug is readily released. It would appear, however, that only 10% of the administered dose reaches the urinary tract in an unmetabolised state (Graber et. al., 1974).

In the 24 hours post-dosing, 45% of radiolabelled oxolinic acid is excreted by man via urine (35%) rather than through the faeces (10%), but none is excreted in as the parent drug (Crew et. al., 1971).

The major metabolites of oxolinic acid in man are summarised in Figure 1 (Graber et. al., 1974). Comparative studies on the excretion of radiolabelled oxolinic acid by rabbit, rat, dog and man revealed that all four species metabolised oxolinic acid in much the same way. However, quantitatively, none of the animal species disposed of oxolinic acid as did man. Of particular interest was the cleavage of the methylenedioxy-ring of oxolinic acid, the first time the cleavage of such a ring had been established in man (Crew et. al., 1971).

Figure 1. Human Metabolism of Oxolinic Acid



Metabolism in Rabbits, Rats and Dogs

In a comparative study of oxolinic acid metabolism, the excretion and metabolic pathways of this substance was studied in rabbits, rats and dogs (Crew et. al., 1971) and the results compared to an earlier study on oxolinic acid metabolism in man (Di Carlo et. al., 1968 a,b). After a single dose of radiolabelled oxolinic acid, at 10 mg/kg the excretion of radioactivity and individual component identification was carried out as thoroughly as possible. The age of this study precludes the accuracy of identification which could be achieved today. The results of this study are summarised in Table 9.

Table 9. Composition of Urinary Radioactivity after Oral Administration of ¹⁴C-Oxolinic Acid (10 mg/kg)*

Species	Time Interval (h)	No. of Animals	Percentage of dose of radioactivity								Total ¹⁴ C
			Unconjugated			Glucuronides			Not known		
			OA	CM	Total	OA	CM	Total			
Rat	0-6	5	1.5	0.3	2.0	2.2	0.9	4.0	4.5	10.5	
Rat	0-6	4	3.4	0.3	3.9	0.4	0.8	1.7	8.1	13.7	
Dog	0-6	1	0.1	0.04	0.3	1.4	0.1	3.2	3.9	7.4	
Man*	0-6	4	0.3	<0.01	0.4	7.8	3.4	12.9	4.6	17.9	
Rat	0-24	5	2.3	0.7	3.3	4.5	2.1	8.9	11.4	23.6	
Rat	0-24	4	5.6	0.9	7.4	1.0	2.0	4.3	19.1	31.4	
Dog	0-24	3	0.3	0.2	1.0	1.8	1.0	7.0	10.5	15.5	
Rabbit	0-24	4	3.8	1.0	4.9	4.9	5.0	21.9	22.1	48.9	

* Dose in man was 1.0 g/subject; OA = oxolinic acid; CM = catechol metabolite (1-ethyl-1,4-dihydro-7-hydroxy-6-methoxy-4-quinolone-3-carboxylic acid)

Metabolism in Fish

Metabolites in bile of rainbow trout

In a study of the metabolic pathway of oxolinic acid in fish (Ishida, 1992), the metabolites of oxolinic acid in the bile of seawater and freshwater trout were compared. Oxolinic acid (OA), two glucuronides of its decomposition products (7-OH-OA-G, 6-OH-OA-G) and oxolinic acid glucuronide (OA-G) were detected in the bile of both groups of trout. The relative concentrations of oxolinic acid and three glucuronides are shown for the two groups of fish in Table 10. Unchanged oxolinic acid was the dominant component 6 h after treatment in both groups. However, 24 h after treatment, oxolinic acid glucuronide was the dominant component, accounting for $66.2 \pm 3.5\%$ for the freshwater trout and $65.7 \pm 7.9\%$ for the seawater trout. The relative amounts of the four compounds in the bile of seawater trout after 24 h were similar to those of freshwater trout (Table 10).

Table 10. Relative Proportions of Oxolinic Acid and its Metabolites in the Bile of Freshwater and Seawater Rainbow Trout after a Single Oral Administration (40 mg/kg)*

Fish	Hours	OA (%)	6-OH-OA-G (%)	7-OH-OA-G (%)	OA-G (%)
Freshwater rainbow trout	6	62.0 ± 30.1	n.d.	n.d.	38.0 ± 30.8
	12	58.1 ± 36.3	n.d.	n.d.	41.9 ± 36.3
	24	28.8 ± 1.2	1.8 ± 1.3	3.4 ± 3.1	66.2 ± 3.5
Seawater rainbow trout	6	50.2 ± 12.1	n.d.	n.d.	49.8 ± 12.1
	12	28.0 ± 8.6	7.0 ± 8.3	9.4 ± 8.1	55.6 ± 8.2
	24	22.4 ± 6.5	4.6 ± 5.5	7.3 ± 8.6	65.7 ± 7.9

* Mean ± SEM (n=4); OA = oxolinic acid; 7-OH-OA-G = 7-hydroxy derivative glucuronide; 6-OH-OA-G = 6-hydroxy derivative glucuronide; OA-G = oxolinic acid glucuronide; n.d. = not detected.

TISSUE RESIDUE DEPLETION STUDIES

General

Tissue residue depletion studies of oxolinic acid based on the use of tritium labelled compounds have seldom been carried out. All investigations published to date use the rate of disappearance of the parent drug to monitor the rate of tissue depletion. Therefore tissue depletion studies and pharmacokinetics of the parent drug are often dealt with in the same report and recommended withdrawal periods are specifically based on the rate of disappearance of parent drug (active metabolites are seldom discussed). Where depletions studies and pharmacokinetics have been discussed in the same report, they are discussed in the Section on pharmacokinetics (*vide supra*).

METHODS OF ANALYSIS FOR RESIDUES IN TISSUES

General

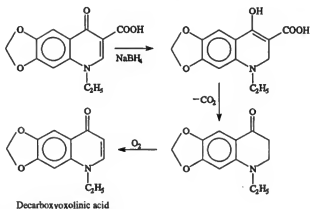
Because of the wide spread use of oxolinic acid, particularly in the aquaculture industry, there are a plethora of publications detailing analytical procedures to quantitate residues of oxolinic acid and related quinolone antimicrobials. All published methods in food matrices measure the concentration of free oxolinic acid in that matrix.

Gas Chromatography - Mass Spectrometry

There appears to be only one direct method for oxolinic acid analysis based on gas chromatography-mass spectrometric detection (Takakuski, 1992). Oxolinic acid and other quinolone acids are extracted with ethyl acetate from fish homogenate buffered at pH 6, back-extracted in bicarbonate, neutralised and again taken up in ethyl acetate. The solvent is evaporated and the residue treated with methanolic sodium borohydride to give decarboxy-derivatives by a process illustrated in Figure 2. The decarboxy-derivatives were then analysed by gas chromatography - mass spectrometry in the SIM mode with a detection limit of 3 ng/g. Recoveries of 80% were obtained for oxolinic acid from fish muscle fortified at the 0.01 ppm level.

This method has been adapted (Pfennig et al., 1993) and used as a confirmatory technique applied to samples where the presence of oxolinic acid was first indicated by HPLC (*vide infra*). It is the only report of the direct confirmation, (based on SIM of 4 ions) of the presence of oxolinic acid residues detected by HPLC in the literature at present. However, an off-line method (Maddock et al, 1983) to establish peak identity in HPLC analysis has also been reported (*vide infra*).

Figure 2. Formation of Decarboxy-oxolinic Acid for GC-MS Determination



Biological Tests

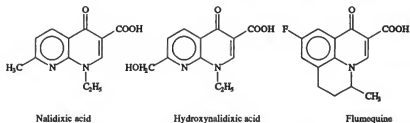
Biological tests based on microbial activity have been developed and applied in a number of studies but suffer from a general lack of sensitivity. Thus a lower limit of detection of only 0.25 µg/mL is achievable with fish plasma or prawn haemolymph (Limpoka et al., 1994).

High Performance Liquid Chromatography (HPLC)

A number of high performance liquid chromatographic methods are fully validated for a range of fish and animal tissues. Many of these methods appear to offer the potential sensitivity and ruggedness to be adaptable to a wide range of biological matrices. These potentially useful methodologies are therefore included in the review of analytical procedures for oxolinic acid.

Several methods for the detection and quantitation of oxolinic acid by HPLC with UV detection have been documented.

Figure 3. Substances Used as Internal Standards in Oxolinic Acid Analysis



An HPLC procedure based on a reverse phase chromatographic separation with UV detection has been successfully developed to monitor oxolinic acid in plasma and tissues of trout and salmon. Hydroxynalidixic acid was utilised as the internal standard for the method. Sample clean-up involved extraction of oxolinic acid into chloroform from tissue homogenates buffered to pH 5.0, evaporation of the solvent and reconstitution of the residue in a pH 6.0 buffer followed by further purification by column chromatography prior to quantitative

determination by reverse-phase HPLC using a buffered methanol with added ion pairing agent (cetyl trimethylammonium bromide) with UV detection at 315 nm. Oxolinic acid could be determined in various tissues, the limit of quantification of the analytical method ranging from 21 ng/g in liver to 68 ng/g in skin, which was the most difficult matrix to analyse (Maddock et al., 1983). The average recovery of hydroxynalidixic acid, the internal standard, was 60-70%. Off-line confirmation of HPLC peak identity could be obtained by collection evaporation of the appropriate HPLC fraction followed by mass spectrometry (Maddock et al., 1983).

An earlier procedure using reverse-phase HPLC separation of oxolinic acid from fish plasma (Rees and Lewis, 1982) proved unsuitable for determination of oxolinic acid residues in tissue samples (Maddock et al., 1983).

Another reverse-phase HPLC procedure for the isolation and determination of oxolinic acid from fish serum employed either liquid or solid phase extraction (Hustvedt et al., 1989). After addition of an internal standard (nalidixic acid), the serum was acidified with hydrochloric acid and extracted with a 1:1 mixture of chloroform and ethyl acetate or applied directly to a solid phase cartridge. The cartridge was washed with sodium hydroxide and the oxolinic acid and internal standard eluted with citrate buffer and methanol. The elution solvent was buffered aqueous methanol containing an ion pairing agent (tetrabutylammonium phosphate) with UV detection at 258 nm. The detection limit for oxolinic acid by this method was 1 ng/mL while the Coefficient of Variation (CV) at 1 µg/mL was 17.5% for the solid phase extraction but only 3% for liquid extraction. The CV for solid phase extraction was lowered to less than 3% by repeated use of a single extraction cartridge.

The simultaneous extraction and determination of oxolinic acid and flumequine in fish tissues by high performance liquid chromatography has also been reported (Rogstad et al., 1989). This study compared the extraction efficiency of oxolinic acid and flumequine by organic solvents and solid phase extraction, respectively. Flumequine was used as an internal standard for the determination of oxolinic acid, whilst oxolinic acid served as the internal standard for the determination of flumequine. HPLC was conducted on a resin column under acidic conditions using a mobile phase of acetonitrile, tetrahydrofuran and aqueous phosphoric acid with fluorescence detection. The limit of quantification in muscle and liver was 0.5 ng/g for oxolinic acid and 2 ng/g for flumequine. The study showed that the efficiency of extraction was lower with a solid phase extraction (SPE) cartridge than with solvent, while the CVs for the analyses were similar for either extraction method. It was concluded that use of SPE did not appear to be more cost effective than solvent extraction. Comparative data for the efficiency of the two extraction methods for fish muscle are shown in Table 11.

A rapid and highly sensitive method for the quantitative determination of oxolinic acid and flumequine in salmon plasma by high-performance liquid chromatography with fluorescence detection has been developed (Samuelsen, 1990). As in the previous method, flumequine was used as an internal standard for the determination of oxolinic acid whilst oxolinic acid served as the internal standard for the determination of flumequine. Protein in the fish plasma was precipitated with zinc sulfate and acetonitrile and, after centrifugation, the supernatant was analysed directly on a C_4 reverse phase column using a complex mixture of solvents buffered to pH 3.2 with oxalic acid. Recoveries were in excess of 90% for both analytes at 50 ng/mL whilst limits of detection were 3 and 5 ng/mL for oxolinic acid and flumequine, respectively.

Two separate methods for the determination of oxolinic acid and flumequine in salmon plasma have been described (Rasmussen et al., 1989a,b). In the first method, the sample is applied to a disposable C_4 solid phase extraction column. Nalidixic acid was added as the internal standard and the analytes eluted from the C_4 column using a mixture of acetonitrile, methanol and 1M phosphoric acid. In the second method the plasma was injected directly onto a polystyrene-divinylbenzene precolumn for sample cleanup. The precolumn was eluted with dilute phosphoric acid until interfering substances were removed followed by elution of the analytes onto the analytical column with the mobile phase. HPLC conditions were common to either cleanup procedure, utilising a resin-based column under acidic conditions with a mobile phase of acetonitrile, tetrahydrofuran and aqueous phosphoric acid with fluorescence detection as previously reported (Rogstad et al., 1989). The limit of determination for the column switching procedure was 5 and 10 ng/mL for oxolinic acid and flumequine, respectively. The recovery and reproducibility of analysis of oxolinic acid by both isolation methods is shown in Table 12.

Table 11. Comparative Data for the Isolation of Oxolinic Acid and Flumequine from Fish Muscle by Solvent Extraction and Solid Phase Extraction (Rogstad et. al., 1989)

Solvent Extraction						
Tissue	Number of Samples	Spike Level ($\mu\text{g/g}$)	Recovery (%)			
			Oxolinic Acid Mean	SD	Flumequine Mean	SD
Muscle) (5 g)	10	0.1	97	3.7		
	6	0.01	97	7.2		
	8	0.5			92	3.8
	6	0.1			89	3.9
Solid Phase Extraction						
Tissue	Number of Samples	Spike Level ($\mu\text{g/g}$)	Recovery (%)			
			Oxolinic Acid Mean	SD	Flumequine Mean	SD
Muscle (5 g)	8	0.1	78.4	2.2		
	8	0.5			77.1	3.8

SD = Standard Deviation

Table 12. The Recovery and Reproducibility of Analysis of Oxolinic Acid in Fish Plasma by Solid Phase Extraction and Column Switching Cleanup Techniques (Rasmussen et. al., 1989a,b)

Conc. of oxolinic acid ($\mu\text{g/mL}$)	Solid Phase Extraction Cleanup Technique		Column Switching Cleanup Technique	
	Mean Recovery (%) (n=6)	CV (%)	Mean Recovery (%) (n=6)	CV (%)
0.05			97.5	2.7
0.5	103.6	4.2	97.2	1.0
1.0	100.1	2.5	98.1	1.1
2.5	100.4	2.8	98.1	1.9
5.0	98.7	1.2	95.7	1.0

The poor adsorption of oxolinic acid by fish has raised the possibility that it could become an environmental contaminant. A reverse phase HPLC method for the determination of oxolinic acid with UV detection has been reported (Pouliquen et. al., 1994) with the object of studying the occurrence, persistence and metabolism of oxolinic acid in seawater, marine sediment and Japanese oyster. Linearity and precision were satisfactory over a concentration range of 0.05-2.5 $\mu\text{g/mL}$ or $\mu\text{g/g}$ with limits of detection and determination of 0.01 and 0.04 $\mu\text{g/mL}$ or $\mu\text{g/g}$ respectively. Recoveries of oxolinic acid from spiked samples were 102.1% from seawater, 68.1% from marine sediment and 88.5% from oyster. The analyte was stable in all matrices at -20°C for 60 days. No analyses of actual environmental samples were reported in this work.

Pfennig et. al. (1993) report the determination of 4 quinolone antimicrobials including oxolinic acid for salmon tissue using acetone extraction, sample cleanup using solvent partition and HPLC determination using a PLRP-S polymer column and fluorescence detection. A similar multi-residue method for several antibiotic classes including oxolinic acid has been published by Nose et. al. (1987) using acetone extraction and alumina cleanup followed by reverse-phase HPLC with UV detection.

APPRAISAL

It has been demonstrated (Ishida, 1990b) that the change in oxolinic acid levels in seawater-acclimatised coho salmon after oral administration was different from that in freshwater coho salmon. It was also found that the change in oxolinic acid levels in seawater coho salmon were similar to those in seawater fishes such as Japanese mackerel, red sea bream, yellowtail, and flounder. Oxolinic acid therefore appears to be retained at higher concentrations and for longer times in the freshwater fishes than in the seawater fish. Experiments with rainbow trout (*vide supra*) supports this conclusion. It is suggested that the difference of the change in oxolinic acid levels after oral administration must be a function of the salinity of the fish's environment (Ishida, 1992). It is also clear that retention of oxolinic acid by some fish species is dependent on water temperature.

Therefore, the utilisation of oxolinic acid in aquaculture must be determined predominantly by its mammalian toxicity rather than by any inherent *in vivo* fish data.

Like many xenobiotic antimicrobial agents, oxolinic acid appears to be benign with respect to human health. The use of this drug in fish husbandry is therefore supported with an appropriate withdrawal time. This withdrawal time must be linked both to the water salinity and the water temperature utilised in fish aquaculture practices.

Maximum Residue Limits

The Committee was not able to set MRLs because no ADI was established. No additional residue data were requested.

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SPIRAMYCIN

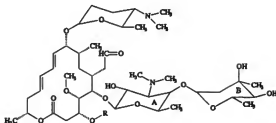
First draft prepared by
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IDENTITY

Chemical name: Spiramycin

Synonyms: CAS 8025-81-8; Foromacidin; Sequamycin;
Selectomycin; Rovamycin; Provamycin

Structural formula:



Molecular formula:	Spiramycin I	(C ₆₀ H ₇₃ N ₃ O ₁₃)-O-R;	R = H
	Spiramycin II	(C ₆₀ H ₇₃ N ₃ O ₁₃)-O-R;	R = COCH ₃
	Spiramycin III	(C ₆₀ H ₇₃ N ₃ O ₁₃)-O-R;	R = COCH ₂ CH ₃
Formula weight:	Spiramycin I	843.07	
	Spiramycin II	885.11	
	Spiramycin III	899.14	

OTHER INFORMATION ON IDENTITY AND PROPERTIES

Pure active ingredient:

Appearance: White or faintly yellowish powder

Melting point: 128-137°C

Solubility: Barely soluble in water, freely soluble in acetone, ethanol and methanol, sparingly soluble in ether

Optical rotation: [α]_D(20) -80° (c = 1 in methanol)

UV_{max}: 231 nm (ethanol)

pH: 8.5-10.5 (0.5% solution)

SUMMARY OF THE 38TH JECFA

METABOLISM

The data available to the Committee indicated that spiramycin could be metabolised into neospiramycin in tissue and that neospiramycin has similar antibacterial activity to the parent drug. There was no structure identification of the neospiramycin metabolite.

TISSUE RESIDUE DEPLETION STUDIES

Radiolabel Residue Depletion Studies

No radiolabel residue depletion studies were submitted by the sponsor for the 38th meeting of the Committee.

Residue Depletion Studies (with Unlabeled Drug)

Cattle

The major study in cattle consisted of an 18 animal study, in which the animals received two intramuscular injections of 100,000 IU/kg body weight (31 mg/kg BW) Suanovil at 48 hour intervals. Groups of three animals were used for each withdrawal period. Male animals were used for the 14 and 21 day withdrawal period; female animals were used for the 28, 35, 42, and 49 day withdrawal periods. Tissues were collected from injection sites, muscle, liver, kidney and fat as noted in Table 1 (Sanders and Delepine, 1990a). Injection site residues were 20.91 and 35.12 µg/g at day 14, 10.23 and 10.30 µg/g at day 21. Residues declined rapidly to 0.47-0.60 µg/g at day 28 and 0.13-0.16 µg/g at day 42. Residues were below the limit of quantification of the liquid chromatographic method by day 49. Neospiramycin residues were reported as spiramycin equivalents using a correction factor of 0.88, the ratio of neospiramycin and spiramycin titers. Concentrations of residues are expressed in µg/g using the WHO standard titer of 3200 IU/mg.

Table 1. Concentrations of Residues in Spiramycin Equivalents (µg/g) in Cattle

Days Post Treatment	Muscle	Liver	Kidney	Fat
14	0.09	0.48	0.47	NS
21	<0.06	0.30	0.17	NS
28	<0.03	0.14	0.05	ND
35	<0.03	<0.12	<0.03	0.05
42	ND	<0.12	<0.03	<0.03
49	ND	<0.06	ND	ND

ND: not detected (<0.015 mg/kg); NS: not sampled.

Although not shown in the Table, residues of neospiramycin were approximately the same concentration as those of spiramycin.

Using 6 cows, residues of spiramycin in milk were determined following intramuscular administration of 30,000 IU/kg body weight (9.3 mg/kg). Milk samples were collected for 25 milkings. Residues were determined using the microbiological agar diffusion method with *Micrococcus luteus* (Moulin and Sanders, 1989a). Results are shown in Table 2.

Table 2. Spiramycin Residues ($\mu\text{g/ml}$) in Milk

Milking	Concentration (mean $\pm$ SD)
1	16.54 $\pm$ 7.50
2	10.00 $\pm$ 3.18
3	5.27 $\pm$ 1.62
4	2.74 $\pm$ 0.90
5	1.50 $\pm$ 0.40
6	1.06 $\pm$ 0.25
7	0.74 $\pm$ 0.57
8	0.57 $\pm$ 0.09
9	0.43 $\pm$ 0.08
10	0.40 $\pm$ 0.05
11	0.32 $\pm$ 0.06
12	0.30 $\pm$ 0.06
13	0.24 $\pm$ 0.10
14	0.19 $\pm$ 0.03
15	0.16 $\pm$ 0.03
16	0.14 $\pm$ 0.03
17	0.09 $\pm$ -
18-25	ND

ND: Not detected ($<0.06 \mu\text{g/ml}$)

Residues of spiramycin were measured in 42 female Friesian/Holstein calves (aged 8 - 15 days) divided into three groups. Group 1 (4 calves) served as controls. Group 2 (22 calves) received 25 mg/kg BW spiramycin per day for seven days in their feed. Group 3 (16 calves) received 25 mg/kg BW spiramycin per day plus 40 mg/kg BW oxytetracycline per day in their feed for seven days. Residues were determined by the microbiological method (Pascal et al., 1990a). Results are shown in Table 3. There was no indication that the admixture with oxytetracycline effected residue concentrations of spiramycin and its metabolites. In addition, oxytetracycline was not found to interfere significantly with the assay of spiramycin residues in tissue.

Table 3. Residues in Calves ($\mu\text{g/g}$) Receiving Spiramycin Alone or in Combination with Oxytetracycline

Days Post Treatment	Muscle	Liver	Kidney	Fat
3	0.2	9.17	13.7	0.15
7	<0.1	3.03	5.3	<0.1
14	<0.1	1.1	1.7	<0.1
24	<0.1	0.2	<0.1	ND
35	ND	<0.1	ND	ND

ND: Not detected

Pigs

Four studies were reported in pigs, one using intramuscular dosing, two using oral administration and one using subcutaneous injection and oral administration. In the intramuscular dosing study, 15 pigs 10-12 weeks old, received 25 mg spiramycin per kg body weight per day for three days. Three animals were used in each withdrawal period. Tissue samples were collected from liver, kidney, muscle, fat, skin, heart and brain. Tissue samples were analyzed using a microbiological assay with *Micrococcus luteus* (May and Baker, 1967). The results are summarized in Table 4.

Table 4. Spiramycin Residues ($\mu\text{g/g}$) in Pigs Receiving 25 mg/kg BW per day Intramuscularly for Three Days

Days Post Treatment	Liver	Kidney	Muscle	Fat	Skin	Heart	Brain
1	9.01	21.59	0.29	0.03	0.11	0.32	0.04
3	2.26	4.75	0.20	0.09	0.20	0.20	<0.025
5	0.68	1.24	0.05	0.05	0.07	0.10	<0.025
7	0.49	0.48	<0.025	0.04	0.04	0.07	<0.025
14	<0.025	0.04	<0.025	<0.025	<0.025	<0.025	<0.025

The two residue studies using oral dosing with spiramycin embonate were conducted in 25-30 kg male and female White X Landrace pigs (Pascal et al., 1990b). In each study, three animals were used for each withdrawal period. The dose in each study is indicated in the tables below. Analysis of spiramycin and its active metabolites in tissues was conducted using the agar diffusion method with *Sarcina lutea* ATCC 9341 as the test organism. In animals receiving spiramycin (16 mg/kg BW/day) combined with sulfadimidine or oxytetracycline, there was no change in the elimination kinetics as compared with animals receiving spiramycin alone at the same dose. Sulfadimidine and oxytetracycline did not interfere significantly with the assay of spiramycin in the tissues. Results are listed in Tables 5 and 6.

Table 5. Tissue Residues ($\mu\text{g/g}$) in Pigs Receiving 16 mg/kg BW per day Spiramycin Embonate for Seven Days

Days Post Treatment	Liver	Kidney	Muscle	Fat
0.5	6.26 $\pm$ 1.26	8.90 $\pm$ 1.75	0.12 $\pm$ 0.01	<0.10
3	1.44 $\pm$ 0.24	1.30 $\pm$ 0.56	<0.10	<0.10
7	0.58 $\pm$ 0.19	0.23 $\pm$ 0.07	<0.10	<0.10
10	<0.30	<0.15	ND	<0.10
15	<0.30	<0.15	ND	ND
20	<0.30	<0.15	ND	ND

ND: Not determined

Table 6. Tissue Residues ($\mu\text{g/g}$) in Pigs Receiving 25 mg/kg BW per day Spiramycin Embonate for Seven Days

Days Post Treatment	Liver	Kidney	Muscle	Fat
7	1.45 $\pm$ 0.40	0.56 $\pm$ 0.11	<0.10	<0.10
10	0.89 $\pm$ 0.35	0.19 $\pm$ 0.05	ND	<0.10
20	<0.30	<0.15	ND	ND

ND: Not determined

Excretion and tissue concentrations of spiramycin were measured in pigs following oral administration (4 animals) or subcutaneous injection (10 animals) (Ferrot and Videau, 1971). Subcutaneous injection gave significantly greater bioavailability than the oral administration at an equivalent dose. However, the residue concentrations in organ tissues and bile resulting from oral administration was considerably higher than reported in other studies, whereas concentrations in muscle by both routes was much lower than in the other tissues and depleted rapidly by comparison to the other studies.

Poultry

One study was reported by Banazet and collaborators (1960). Thirteen broiler chickens approximately 1.2 kg each, were treated with 300 ppm spiramycin in the feed for 10 days. The average daily intake was calculated to be 43 mg per bird. Samples of liver and muscle were analyzed using a microbiological assay using *Micrococcus luteus* ATCC 9341. The results are noted in Table 7.

Table 7. Tissue Residues ($\mu\text{g/g}$) in Broilers Receiving 300 ppm Spiramycin in Feed for 10 Days

Days Post Treatment	Liver	Muscle
0	3.78	0.19
1	1.67	0.08
3	0.89	0.04
5	0.21	<0.02
8	<0.02	<0.02

Note: The assay had a sensitivity of 0.02 $\mu\text{g/g}$

METHODS OF ANALYSIS FOR RESIDUES IN TISSUE

Microbiological and high performance liquid chromatography methods have been reported for the determination of spiramycin residues in plasma, serum, tissues and milk.

The microbiological agar gel diffusion method uses *Micrococcus luteus* ATCC 9341 as the test organism. Tissue samples are extracted and purified prior to adding to the test organism. For serum, samples are diluted with phosphate buffer as needed. Agar plates are prepared using Difco #9 medium and seeded with the test organism. Standards are prepared in blank serum. Cylinders are placed on the seeded plates and 200 μl of material (sample or standard) is added. The method has a sensitivity of approximately 0.1 IU/ml (about 0.03 $\mu\text{g/g}$) (Huet et al., 1988).

Milk samples are prepared in much the same way as serum samples. Milk samples may be analyzed directly or diluted with phosphate buffer as necessary. Plates are prepared as noted above. The test samples (250 μl) are placed in the test cylinders and incubated. Zones of microbial inhibition are measured and the concentration of spiramycin determined by extrapolation with known samples. The quantification limit in milk is 0.2 IU/ml (about 0.06 $\mu\text{g/g}$) (Moulin and Sanders, 1989b).

High performance liquid chromatography (HPLC) has been used to measure spiramycin residues in plasma (Mourout and Sanders, 1988). Test samples and standards added to blank plasma are processed through C2 cartridges using 4% acetonitrile. A 5u reverse phase RP 8 column with a RP 8 precolumn is used for separation of analytes. Samples are eluted with 0.5% sulfuric acid and acetonitrile (79:21, v/v). Detection is by UV, using 231 nm. The method is linear from 0.2 - 104 IU/ml (0.06 - 32 $\mu\text{g/ml}$) with average recoveries of about 85%. The limit of quantification is 0.2 IU/ml (0.06 $\mu\text{g/ml}$).

Tissues are also analyzed for spiramycin and neospiramycin residues by reverse phase HPLC. Tissue samples and standards prepared in blank tissue are extracted using chloroform, and the extracts processed through solid phase extraction cartridges using chloroform. A 5u reverse phase PR 8 column is used as noted above for separation. Samples are eluted with 0.5% sulfuric acid in acetonitrile (80:20 v/v) and detection of residues is monitored with a UV detector using 231 nm. The method is linear for concentrations between 0.2 - 10 IU/g (0.06-3 $\mu\text{g/g}$) with recovery of spiramycin from extracted standards of 76, 85, and 97% for muscle, liver and kidney, respectively. Recovery for neospiramycin is 83, 72, and 80% respectively, for the three tissues. The quantification limit was 0.1 IU/g (0.03 $\mu\text{g/g}$) in muscle and kidney and 0.2 IU/g (0.06 $\mu\text{g/g}$) in liver. The limit of detection was estimated to be 0.05 IU/g (0.015 $\mu\text{g/g}$) (Sanders and Delepine, 1990b).

APPRAISAL

In the assessment on MRL's for spiramycin, the Committee took into consideration the temporary ADI and recommended temporary MRL's for liver, kidney, and muscle tissue in pigs and cattle, and in milk. No MRL was assigned for fat because sufficient data were not available. The Committee requested results on two studies for evaluation in 1994:

1. Radiometric studies on the concentrations of spiramycin and its metabolites as proportions of the total residues in edible tissues of cattle, pigs and poultry.
2. Studies on the pharmacokinetics of spiramycin residues in the fat of cattle and pigs and the edible tissues of poultry.

1994 DATA SUPPLEMENT

RESIDUE STUDIES OF SPIRAMYCIN AND ITS METABOLITES IN EDIBLE TISSUES

Instead of radiometric studies, the sponsor presented extensive studies using microbiological and HPLC methods deemed appropriate to assess residues of spiramycin and its metabolites as proportions of the total residues in edible tissues of the target species. The HPLC method, as noted above, is specific for parent drug and its primary metabolite, neospiramycin. The bioassay indicates the presence of microbiologically active metabolites. The hypothesis of the studies presented is that all spiramycin metabolites possess a certain amount of microbiological activity and that the microbiological method is representative of the total residues (metabolites) in biological fluids and tissues. The original spiramycin submission estimates the microbiological activity of neospiramycin at 88% of spiramycin titre.

Sanders and collaborators (1991) studied residues in plasma from cattle administered Spiramycin intravenously using HPLC and microbiological assays on the same plasma samples. The area under the curve (AUC) was calculated from the microbial inhibition assay and results compared to the HPLC data. The ratio of AUC versus HPLC was estimated at about 1.2:1, indicating the presence of microbiologically active metabolites in plasma. Data presented in the study indicates that approximately 70% of the activity is due to spiramycin at post treatment times up to 96 hours.

Similar studies were reported in cattle tissues. Animals received two intramuscular doses of 100,000 IU/kg (approximately 32 mg/kg) at 48 hour intervals. Microbiological residues were determined using *Micrococcus luteus* (Sarcina luteus) ATCC 9341 (Huet et al., 1988). Spiramycin and neospiramycin residues were measured by reverse phase HPLC (Sanders and Delepine, 1990a; Sanders et al., 1990). Neospiramycin was unambiguously identified in cattle muscle by co-elution with an analytical standard with structure confirmed by mass spectrometry. A comparison of the concentrations in the same samples is presented in Table 8. Ratios of the two sets of results are also tabulated. Each data point represents the mean of three tissue samples.

Table 8. Residues of Spiramycin and Neospiramycin ($\mu\text{g/kg}$) in Cattle Tissue Determined by HPLC and Total Active Metabolites by *Micrococcus luteus* ATCC 9341

Days Post Treatment	Assay Method	Liver	Kidney	Muscle	Fat
14	HPLC	480	470	90	NS
	ATCC 9341	718	742	66	NS
	Ratio (%)	67	63	100	-
21	HPLC	300	170	<60	NS
	ATCC 9341	220	172	53	NS
	Ratio (%)	100	99	100	-
28	HPLC	140	<50	<30	<30
	ATCC 9341	151	85	<25	85
	Ratio (%)	93	59	100	35
35	HPLC	<120	<30	<30	50
	ATCC 9341	118	48	27	69
	Ratio (%)	100	62	100	72

NS: Not sampled.

Values for the HPLC assay represent the sum of spiramycin and neomycin residues.
Ratios expressed as %: (spiramycin + neospiramycin/total active metabolites) x 100

Comparing residue concentrations by the two methods indicates that spiramycin and neospiramycin represent the majority of the total active residues in cattle tissue. In muscle tissue, spiramycin and neospiramycin are the only detectable residues in muscle tissue. Similarly, ratios in liver indicate that these two analytes represent 67%, 100%, 93%, and 100% of total active residues at days 14, 21, 28 and 35, respectively. In kidney, the results are somewhat more variable, with ratios of spiramycin and neospiramycin ranging from 60% to 100% of total active metabolites. Data in fat is limited to results from 28 and 35 days only, with ratios of 35% and 72%, respectively. Results from cattle plasma and tissues are in general agreement with the pharmacokinetic properties of spiramycin in cattle (i.e., large body distribution and a long elimination half-life) (Sanders et al., 1992). The presence of minor quantities of unknown active metabolites in the liver and kidney may be accounted for by a further metabolic transformation of neospiramycin to polar metabolites (noted later in pig and poultry studies) by formation of adducts of neospiramycin with L-cysteine. The proposed metabolism of spiramycin in cattle will be summarized following the discussion of the poultry metabolism studies, since data strongly suggest a similar metabolic scenario.

Poultry

Studies on spiramycin metabolism in chickens were carried out using two medicated diets of 10 g (the therapeutic dose for growth promotion) and 100 g of spiramycin per ton of food for about 60 days (Jolles and Terlain, 1968). Muscle, liver, and kidney tissues were sampled at the end of the treatment period. The withdrawal time was not reported by the authors. Residues were analyzed by thin layer chromatography (TLC) following solvent extraction, with detection by bioautography. Detection limits for this assay was about 0.01 and 0.02 µg/g for spiramycin and neospiramycin, respectively. Results indicate that these two analytes were the major microbiologically active residues in all three tissues. The polar metabolites were tentatively identified as conjugates or bound forms of neospiramycin. Jolles and Terlain indicate that these metabolites appear to be neither glucuro- nor sulfoconjugates. Neospiramycin could be partially regenerated from the conjugates on treatment of the residues at 50 to 60°C with organic solvents or storage at room temperature for longer periods of time. Results are summarized in Table 9.

Table 9. Residues of Spiramycin (µg/g) in Chicken Tissues

Tissue	Spiramycin	Neospiramycin	Polar Derivatives
Muscle	0.010 - 0.015	<0.010	ND
Liver	1.0 - 1.3	0.8 - 1.0	<2.3
Kidney	0.12 - 0.15	0.10 - 0.12	<2.7

ND: Not detected

Data represents the highest dose group (100 g per ton)

Jolles and Terlain (1968) estimated concentrations of residues by comparison with fortified blank tissue with known quantities of each analyte, however, this could not be done with the polar metabolites. The authors concluded that the inhibition area attributed to the polar substances on *Bacillus subtilis* bioautographs was smaller than the sum of the inhibition zones of the other two analytes. The polar derivatives, based on this observation and the data in Table 9 indicate that the polar metabolites do not contain more than 50% of the total residues.

A major residue study in poultry is described by Bosc and collaborators (1993) and Mignot and collaborators (1993). Bosc and collaborators conducted the animal dosing studies by addition of Suanovil 50 in the drinking water at 0.8 g/l for three days. Mignot and collaborators determined spiramycin and neospiramycin residues in plasma, muscle, liver, kidney, and skin with fat using an HPLC method. Residues were measured at 5, 10, 15 and 20 days after the last treatment. Six birds were used in each sampling period. Mignot and collaborators (1993) measured residues in liver in five samples at day 5 but were only able to detect residues in two samples at day 10. In kidney, residues were measured in only two samples at day 5; in the four remaining samples spiramycin was detected but residues were below the limit of quantification. Concentrations of spiramycin and neospiramycin were most often below the limit of quantification in muscle and skin with fat at day 5 after the last treatment. Residues were highest in liver but were below the limit of quantification by day 15; for kidney, by day 10 after the last treatment. Results are summarized in Table 10.

Table 10. Residues of Spiramycin and Neospiramycin in Poultry Tissues (ng/g)

Days Post Treatment	Muscle		Liver		Kidney		Skin with fat	
	SP	NSP	SP	NSP	SP	NSP	SP	NSP
5	55 (n=2)	+	699 (n=5)	579	280 (n=2)	+	99 (n=3)	+
10	+	+	423 (n=2)	491	+	+	+	+
15	+	+	+	+	+	+	+	+
20	+	+	+	+	+	+	+	+

SP is spiramycin; NSP is neospiramycin.

Limit of quantification for spiramycin and neospiramycin was 50, 100, 200, and 75 ng/g, respectively for muscle, liver, kidney and skin with fat. A * + * indicates results below the limit of quantification.

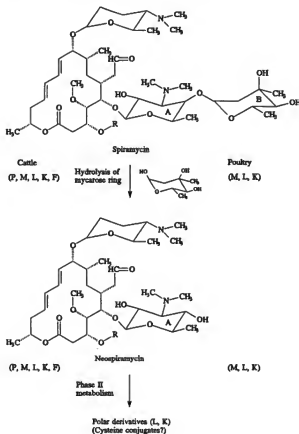
Based on the collective data of these studies, spiramycin metabolism occurs by hydrolysis to neospiramycin by loss of the mycarose moiety. Neospiramycin residues are present at lower concentrations than parent drug and polar residues were noted by the authors in liver, kidney and fat. Total residues represent the microbiologically active residues. The authors noted that the proportion of spiramycin and neospiramycin versus total microbiologically active residues in skin and fat is a conservative estimate as an additional safety margin. These results are summarized in Table 11.

Table 11. Residues of Spiramycin and Neospiramycin ($\mu\text{g/g}$) Versus Total Residue in Edible Tissues of Chicken

Tissue	Ratio (%) (Spiramycin + Neospiramycin/Total Residues) x 100
Muscle	100
Liver	50
Kidney	50
Fat	50

Based on these metabolism studies, the proposed metabolic pathway for cattle and poultry is noted in Figure 1.

Figure 1. Proposed Metabolic Pathway for Spiramycin in Cattle and Poultry



P = plasma, M = muscle, L = liver, K = kidney, F = fat

Pigs

Mourier (1993) conducted a metabolism study in pigs using spiramycin embonate to evaluate and identify the polar metabolites in pig liver. The initial work was an *in vitro* study to identify the principal biotransformations. Results indicated that L-cysteine (present in relatively large quantities in pig liver), reacts with the macrocycle aldehyde, resulting in the formation of a thiazolidine carboxylic acid. Additional studies showed that only spiramycin derivatives whose aldehyde function is modified do not undergo this transformation. In a subsequent study with pigs treated orally with 50 mg/kg BW per day for seven days, and using an HPLC assay method (detection limit of 0.2 µg/g) six spiramycin metabolites were detected. (See page 1 of this monograph to identify the structure of spiramycin I and III.)

Spiramycin I	0.2 µg/g
Spiramycin III	0.2 µg/g
Spiramycin I with cysteine	6.4 µg/g
Spiramycin III with cysteine	4.1 µg/g
Neospiramycin I with cysteine	1.2 µg/g
Neospiramycin III with cysteine	1.0 µg/g
Total Spiramycin residues	13.1 µg/g

This study provides strong support for the proposed metabolism noted in Figure 1. It suggests, though there is not metabolism data in other pig tissues, that the metabolism shown in Figure 1 is representative of metabolism in pig muscle and kidney.

A definitive study of spiramycin residues in pig tissues was reported by Cuypers and collaborators (1994). Twelve castrated piglets weighing 15-18 kg received spiramycin embonate feed dosed at 450 ppm. Daily ingested doses were measured at 21-23 mg/kg live weight of spiramycin base. Groups of four animals were analyzed at each withdrawal time. Spiramycin residues were analyzed by HPLC and the microbiological assay using *Micrococcus luteus* ATCC 9341. The HPLC method had a limit of quantification of 0.1 µg/g, and analyte extraction recovery of 89%. The tissue concentrations represent spiramycin and its metabolites in pig liver receiving 22 mg/kg spiramycin embonate per day for seven days. At all three withdrawal times, the highest residue concentrations were the cysteine derivative of spiramycin I and III. Residues of neospiramycin and its cysteine derivatives were very low. Results are summarized in Table 12. These data support the hypothesis that spiramycins are metabolized to neospiramycins in pig liver to only a slight extent. Spiramycin and neospiramycin combine readily with L-cysteine to yield thiazolidine derivatives.

Table 12. Residues of Spiramycin and Metabolites (µg/g) in Pig Liver Measured by the HPLC and Microbiological Methods

Days Post Treatment	Residues by HPLC	Microbiologically Active Residues
0	5.0	5.2
3	0.9	1.3
10	0.1	<0.2

METHODS OF ANALYSIS FOR RESIDUES IN TISSUES

Both microbiological and HPLC methods are available for residues of spiramycin in cattle tissues. The microbiological method is based on the agar diffusion method with *Sarcina lutea* ATCC 9341 as the test organism. An organic extraction and purification process is employed prior to application of the microbiological assay (Pascal, et al., 1990). The HPLC method for the determination of spiramycin and neospiramycin has been referenced previously (Sanders and Delepine, 1990b). A microbiological method is available for residue analysis of milk samples (Moulin and Sanders, 1989b) and employs the same agar diffusion method noted above. The limit of quantification is 62 µg/l of total residues.

For pig tissues, a microbiological method is available for residue studies virtually identical to those referenced above (Pascal, et al., 1990). An HPLC method is available for liver tissue only.

For poultry, an HPLC method for residue analysis in edible tissues has been reported (Mignot, et al., 1993). It is suitable for both spiramycin and neospiramycin.

Tables 13 and 14 summarize the limits of quantification for cattle, pigs and chickens tissues, of analytes of interest.

Table 13. Limits of Quantification for Residues of Spiramycin ($\mu\text{g/kg}$)

Species	Method	Muscle	Liver	Kidney	Fat	Milk
Cattle	ATCC 9341	100	100	100	100	62
	HPLC	30	62.5	30	47	
Pig	ATCC 9341	100	300	150	100	
Chicken	HPLC	50	100	200	75	

Table 14. Limits of Quantification for Residues of Neospiramycin ($\mu\text{g/kg}$)

Species	Method	Muscle	Liver	Kidney	Fat	Milk
Cattle	ATCC 9341	100	100	100	100	62
	HPLC	25	50	15	30	
Pig	ATCC 9341	100	300	150	100	
Chicken	HPLC	50	100	200	75	

The *Micrococcus luteus* ATCC 9341 residue assay measures total microbiological activity. The estimated microbiological activity of neospiramycin is 88% of spiramycin activity.

Although the *Micrococcus luteus* ATCC 9341 methodology has ample data to verify its use for measuring residues of spiramycin biologically active residues, it is not an unambiguous method for use in a regulatory control program. The USDA's Food Safety and Inspection Service Microbiology Laboratory Guidebook uses this same test organism, as well as other test organisms, to specifically identify several classes of antibiotics, including penicillin, streptomycin, erythromycin and neomycin. To specifically identify the analyte of interest requires development of a fingerprint profile of each class of antibiotics with a set of several test organisms.

APPRAISAL

Residues of spiramycin in cattle are highest in liver tissue following intramuscular injection at all withdrawal times up to 49 days, with quantifiable residues up to 28 days. With oral treatment, the results indicate that residues in kidney are somewhat higher than in liver tissue for at least 14 days and comparable at longer withdrawal times. In pigs following intramuscular treatment residues are approximately two fold higher in the kidney through the first seven days post treatment, but are below the limits of quantitation by day 14.

With pigs receiving medicated feed, residues are similar in the liver and kidney at three days withdrawal but are higher in the liver by day seven. Residues tend to deplete more rapidly in pigs than in cattle and that may be due to the relatively high amounts of L-cysteine in pigs resulting in more extensive thiazolidine carboxylic acid derivative formation. The data suggest that liver is the most reasonable target tissue for residue analysis. The parent drug and the neospiramycin or the microbiologically active residues are suitable as the marker residue.

In poultry, the residue data at withdrawal times of 5, 10, 15 and 20 days were studied by Boec and Mignot and collaborators (1993). Concentrations of residues measured by an HPLC method were often below the limit of quantification in muscle and skin with fat even at day 5 after the last treatment. Residues were higher in liver tissue than in kidney, while residues appear to be low (ng/g) at all withdrawal times. On this basis, liver is indicated as the target tissue for poultry and spiramycin would be the appropriate marker residue. A microbiological method is available (Benazet, et al., 1960) using *Micrococcus luteus* ATCC 9341 as the test organism.

With the exception of muscle tissue, total microbiological activity is greater than residues detected as spiramycin or neospiramycin by HPLC, indicating other metabolites exhibiting biological activity are likely to be present. In cattle, for example, HPLC measured residues are 59-100% of the residues determined by the microbiological assay in liver and kidney tissue. In chickens, residues by HPLC in liver, kidney and fat are conservatively estimated at about 50% of microbiologically active residues. In pigs, the residues by HPLC in liver are 71-96 % of microbiologically active residues for spiramycin I and III, respectively, depending on the withdrawal time.

Maximum Residue Limits

Based on the ADI of 0-50 µg per kg body weight established by the Committee using microbiological data, the permitted daily intake of spiramycin and its antimicrobially active residues would be 3000 µg for a 60-kg person.

In reaching its decision on MRLs, the Committee also took into account the available residue and metabolism data on spiramycin and neospiramycin, the percentage of the total antimicrobial activity representing these residues, and the limits of quantification for the HPLC and microbial methods.

The Committee calculated the minimum residue levels that could be considered as a basis for MRL calculations using twice the limits of quantification for spiramycin and neospiramycin residues in cattle, pigs and chickens (Table 15).

Table 15. Minimum Residue Levels that could be considered as a basis for MRL calculations for Spiramycin (µg/kg) in cattle, pigs and chickens*

Species	Analytical method	MRL levels				
		Muscle	Liver	Kidney	Fat	Milk
Cattle	Agar diffusion <i>M. luteus</i> ATCC 9341	200	200	200	200	124
	HPLC	60 ^b 50 ^c	124 ^b 100 ^c	60 ^b 30 ^c	94 ^b 60 ^c	26 ^b 12 ^c
Pigs	Agar diffusion <i>M. luteus</i> ATCC 9341	200	600	300	200	NA
Chickens	HPLC	100 ^b	200 ^b	400 ^b	150 ^b	NA
		100 ^c	200 ^c	400 ^c	150 ^c	NA

NA: not applicable; * based on the two-fold quantification limits of the analytical methods, for the agar diffusion method, values refer to the sum of residues of spiramycin and neospiramycin; ^b values refer to spiramycin residues; ^c values refer to neospiramycin residues

These values were not adjusted to account for the percentage of antimicrobial activity represented by the sum of spiramycin and neospiramycin residues. In cattle these two residues accounted for about 100% of the total antimicrobial activity in muscle, 85% in liver, 70% in kidney and 50% in fat. For pig tissues, the residues were determined by a validated microbial method. A validated chemical method for the determination of spiramycin and neospiramycin was not available, hence it was not possible to estimate the percentage of the total antimicrobial activity due to spiramycin and neospiramycin residues. In chickens, these two residues accounted for about 100% of total antimicrobial activity in muscle, and 50% in liver, kidney and fat.

The recommended MRLs for spiramycin in cattle, pigs and chickens are shown in Table 16. For cattle and chickens the MRLs are expressed as the sum of spiramycin and neospiramycin. For pigs, the MRLs are expressed as spiramycin equivalents and are temporary for liver, kidney and fat based on antimicrobial

activity, pending the availability of a validated chemical method and the estimation of the percentage of antimicrobially active residues represented by spiramycin and neospiramycin in these tissues.

Table 16. Recommended MRLs for Spiramycin ($\mu\text{g/kg}$) in cattle, pigs and chickens*

Species	MRL				
	Muscle	Liver	Kidney	Fat	Milk ^b
Cattle	100	300	200	300	100
Pigs	200 ^c	600 ^{c,d}	300 ^{c,d}	200 ^{c,d}	NA
Chickens	200	400	800	300	NA

* Expressed as the sum of spiramycin and neospiramycin, if not otherwise stated;

^b Expressed as $\mu\text{g/l}$; ^c Expressed as spiramycin equivalents; ^d Temporary MRL;
NA: not applicable

If these values are used for MRLs, the theoretical maximum daily intake of residues of spiramycin and neospiramycin from cattle and pigs would be less than that from chickens. The theoretical maximum daily intake of antimicrobially active residues from chickens is 250 μg , based on a daily food intake of 300 g of muscle, 100 g of liver, and 50 g of each of kidney and fat. For bovine milk, theoretical maximum daily intake of spiramycin and neospiramycin residues is 150 μg , based on a daily consumption of 1.5 l.

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SUMMARY OF JECFA EVALUATIONS OF VETERINARY DRUG RESIDUES FROM THE 32ND MEETING TO THE PRESENT

The attached table summarizes the veterinary drug evaluations conducted by JECFA at the 32nd (1989), the 34th (1989), the 36th (1990), the 38th (1991), the 40th (1992) and the 42nd (1992) Meetings. These meetings were devoted exclusively to the evaluation of veterinary drug residues in foods. Please see Reports of those meetings, published in WHO Technical Report Series.

Some notes regarding the Table:

- The "Status" column refers to the ADI and indicates if "No" ADI was established, if a full ADI was given, or if the ADI is Temporary (TE).
- Where an MRL is temporary, it is so indicated by "TE".
- Several compounds have been evaluated more than once. The data given is for the most recent evaluation.

Substance	ADI ($\mu\text{g/kg bw}$)	ADI status	JECFA	MRL ($\mu\text{g/kg}$)	Tissue	Species	Marker residue (MR)
Albendazole	0-50	Full	34 (1989)	100 ¹ 5000 ¹	Muscle, fat, milk Liver, kidney	Cattle, sheep	2-Aminobenzimidazole sulfone, MR in milk needs to be identified
Azaperone	0-3	TE	43 (1994)	60 TE 100 TE	Muscle, fat Liver, kidney	Pigs	Sum of azaperone and azaperol
Benzydipenicillin	30 $\mu\text{g/person/day}$	Full	36 (1990)	50 4	Muscle, liver, kidney Milk	All species	Parent drug
BST	Not specified	Full	40 (1992)	Not specified	Muscle, liver, kidney, fat, milk	Cattle	

Carbadox	Limited acceptance	Full	36 (1990)	30 ² 5 ¹	Liver Muscle	Pigs	Quinoxaline-2- carboxylic acid
Carazolid	0-0.1	Full	43 (1994)	5 ¹ 25	Muscle, fat/skin Liver, kidney	Pigs	Parent drug
Chloramphenicol	None	No	42 (1994)	No MRL			
Chlorpromazine	None	No	38 (1991)	No MRL			
Cloxacil	0-30	Full	36 (1990) 40 (1990)	1000 3000 1500 5000 2000	Muscle, liver Kidney, fat Muscle, liver Kidney Fat	Cattle Sheep	Parent drug
Dexamethasone	0-0.015	Full	42 (1992) 43 (1994)	0.5 TE 2.5 TE 0.3 µg/l TE	Muscle, kidney Liver Milk	Cattle, horses, pigs Cattle	Parent drug
Dihydrostreptomycin & streptomycin	0-30 (Group ADI)	TE	43 (1994)	500 TE 1000 TE 200 µg/l TE	Muscle, liver, fat Kidney Milk	Cattle, pigs, chickens, sheep Cattle	Sum of dihydrostreptomycin and streptomycin
Dimetridazole	None	No	34 (1989)	No MRL			
Diminazene	0-100	Full	42 (1994)	500 12000 6000 150 µg/l	Muscle Liver Kidney Milk	Cattle	Parent drug
Enrofloxacin	0-0.6	TE	43 (1994)	No MRL			
Estradiol-17β	Unnecessary	Full	32 (1987)	Unnecessary		Cattle	

Fenbendazole	0-10	TE	38 (1991)	100 ^a TE 500 ^a TE 100 ^a µg/l TE	Muscle, kidney, fat Liver Milk	Cattle, sheep, pigs Cattle	MRL value is the sum of fenbendazole, and oxfendazole, and oxfendazole sulfone, calculated as oxfendazole sulfone equivalents
Fenbendazole	0-25	TE	38 (1991)	100 ^a TE 500 ^a TE 100 ^a µg/l TE	Muscle, kidney, fat Liver Milk	Cattle, sheep, pigs	MRL value is the sum of fenbendazole, oxfendazole, and oxfendazole sulfone, calculated as oxfendazole sulfone equivalents
Flubendazole	0-12	Full	40 (1992)	10 200 500 400	Muscle, liver Muscle Liver Eggs	Pigs Poultry	Parent drug
Flumequine	None	No	42 (1994)	No MRL			
Furazolidone	None	No	40 (1992)	No MRL			
Gentamicin	0-4	TE	43 (1994)	100 TE 200 TE 1000 TE 100 µg/l TE	Muscle, fat Liver Kidney Milk	Cattle, pigs Cattle	Parent drug
Iprnidazole	None	No	34 (1989)	No MRL			
Isometamidium	0-100	Full	40 (1992)	100 500 1000	Muscle, fat, milk Liver Kidney	Cattle	Parent drug
Ivermectin	0-1	Full	40 (1992)	100 40 15 20	Liver Fat Liver Fat	Cattle Other species	H ₂ P ₁₂ H ₁₈

Levamisole	0-6	Full	42 (1992)	10 100	Muscle, kidney, fat Liver	Cattle, sheep, pigs and poultry	Parent drug
Metronidazole	None	No	34 (1989)	No MRL			
Neomycin	0-30	TE	43 (1994)	500 TE 5000 TE 500 TE 500 µg/l TE	Muscle, liver, fat Kidney Eggs Milk	Cattle, chickens, ducks, goats, pigs, sheep, turkeys Chickens Cattle	
Nitrofurazone	None	No	40 (1992)	No MRL			
Olaquinox	Limited acceptance	TE	42 (1994)	No MRL but 4 µg/kg of MQCA (TE) is consistent with GVP	Muscle	Pigs	MQCA
Oxfendazole	0-4	TE	38 (1991)	100* TE 500* TE 100* µg/l TE	Muscle, kidney, fat Liver Milk	Cattle, sheep, pigs Cattle, sheep, pigs Cattle	MRL value is the sum of fenbendazole, oxfendazole, and oxfendazole sulfone, calculated as oxfendazole sulfone equivalents
Oxolinic acid	None	No	43 (1994)	No MRL			
Oxytetracycline	0-3	Full	36 (1990)	100 300 600 10 100 200	Muscle Liver Kidney Fat Milk Eggs	All species	Parent drug
Progesterone	Unnecessary	Full	32 (1987)	Unnecessary		Cattle	
Propionylpromazine	None	No	38 (1991)	No MRL			

Ractopamine	Nose	No	40 (1992)	No MRL				
Ronidazole	Withdrawn	No	42 (1994)	No MRL				
Spectinomycin	0-40	Full	42 (1994)	300 TE 2000 TE 5000 TE 500 TE 200 µg/l TE	Muscle Liver Kidney Fat Milk	Cattle, pigs, and chicken	Parent drug	
Spiramycin	0-50	Full	43 (1994)	100" 200" 300" 600 TE" 400" 200" 300 TE" 800" 300" 200 TE" 300" 100 µg/l"	Muscle Muscle Muscle Liver Liver Liver Kidney Kidney Kidney Fat Fat Fat Milk	Cattle Pigs Chickens Cattle Pigs Chickens Cattle Pigs Chickens Cattle Pigs Chickens Cattle	"Sum of spiramycin and neospiramycin " "Expressed as spiramycin equivalents (antimicrobially active residues)	
Streptomycin (see dihydrostreptomycin)								
Sulfadiazine	0-50	Full	42 (1994)	100 25 µg/l	Muscle, liver, kidney, fat Milk	Cattle, sheep, pigs and poultry Cattle	Parent drug	
Sulphathiazole	None	No	34 (1989)	No MRL				
Testosterone	Unnecessary	Full	32 (1987)	Unnecessary		Cattle		
Tiabendazole	0-100	Full	40 (1992)	100 100	Muscle, liver, kidney, fat Milk	Cattle, pigs, goats and sheep Cattle, goats	Sum of tiabendazole and 5- hydroxytiabendazole	

Trenbolone acetate	0-0.02	Full	34 (1989)	2 as MR 10 as MR	Muscle Liver	Cattle	β -Trenbolone α -Trenbolone
Triclabendazole	0-3	Full	40 (1992)	200 300 100 100	Muscle Liver, kidney Fat Muscle, liver, kidney, fat	Cattle Sheep	5-Chloro-6-(2',3'- dichlorophenoxy)- benzimidazole-2-one
Tylosin	None	No	38 (1991)	No MRL			
Zenanol	0-0.5	Full	32 (1987)	2 ¹ 10 ⁴	Muscle Liver	Cattle	Parent drug

¹Parent drug equivalents; ²As quinoxaline-2-carboxylic acid (QCA); ³The Committee noted that the concentration of carazotol at the injection site may exceed the ADI which is based on the acute pharmacological effects of carazotol; ⁴Group MRL for febantel, fenbendazole and oxfendazole, singly or in combination, as oxfendazole sulfone equivalents

RECOMMENDATIONS ON COMPOUNDS EVALUATED BY THE 43RD JECFA

Substance	ADI $\mu\text{g}/\text{kg}$ bw	MRLs
β-Adrenoceptor-blocking agent		
Carazolol	0-0.1	Muscle & fat/skin (pigs): 5 $\mu\text{g}/\text{kg}^{\text{a,b}}$ Liver & kidney (pigs): 25 $\mu\text{g}/\text{kg}^{\text{a,b}}$
Antimicrobial agents		
Dihydrostreptomycin & streptomycin	0-30 ^d	Muscle, liver & fat (cattle, pigs, chickens & sheep): 500 $\mu\text{g}/\text{kg}^{\text{e,f}}$ Kidney (cattle, pigs, chickens & sheep): 1000 $\mu\text{g}/\text{kg}^{\text{e,f}}$ Milk (cattle): 200 $\mu\text{g}/\text{l}^{\text{e,f}}$
Enrofloxacin	0-0.6 ^e	No MRLs recommended ^g
Gentamycin	0-4 ^e	Muscle & fat (cattle & pigs): 100 $\mu\text{g}/\text{kg}^{\text{a,g}}$ Liver (cattle & pigs): 200 $\mu\text{g}/\text{kg}^{\text{a,g}}$ Kidney (cattle & pigs): 1000 $\mu\text{g}/\text{kg}^{\text{a,g}}$ Milk (cattle): 100 $\mu\text{g}/\text{l}^{\text{a,g}}$
Noomycin	0-30 ^e	Muscle, liver & fat (cattle, chickens, ducks, goats, pigs, sheep & turkeys): 500 $\mu\text{g}/\text{kg}^{\text{a,b}}$ Kidney (cattle, chickens, ducks, goats, pigs, sheep & turkeys): 5000 $\mu\text{g}/\text{kg}^{\text{a,b}}$ Eggs (chickens): 500 $\mu\text{g}/\text{kg}^{\text{a,b}}$ Milk (cattle): 500 $\mu\text{g}/\text{l}^{\text{a,b}}$
Oxolinic acid	No ADI allocated ^f	No MRLs recommended ^g
Spiramycin	0-50	Muscle (cattle): 100 $\mu\text{g}/\text{kg}^{\text{h}}$ Muscle (pigs & chickens): 200 $\mu\text{g}/\text{kg}^{\text{h}}$ Liver (cattle): 300 $\mu\text{g}/\text{kg}^{\text{h}}$ Liver (pigs): 600 $\mu\text{g}/\text{kg}^{\text{a,b}}$ Liver (chickens): 400 $\mu\text{g}/\text{kg}^{\text{h}}$ Kidney (cattle): 200 $\mu\text{g}/\text{kg}^{\text{h}}$ Kidney (pigs): 300 $\mu\text{g}/\text{kg}^{\text{a,b}}$ Kidney (chickens): 800 $\mu\text{g}/\text{kg}^{\text{h}}$ Fat (cattle): 300 $\mu\text{g}/\text{kg}^{\text{h}}$ Fat (pigs): 200 $\mu\text{g}/\text{kg}^{\text{a,b}}$ Fat (chickens): 300 $\mu\text{g}/\text{kg}^{\text{h}}$ Milk (cattle): 100 $\mu\text{g}/\text{l}^{\text{h}}$
Glucocorticosteroid		
Dexamethasone	0-0.015 ⁱ	Muscle & kidney (cattle, horses & pigs): 0.5 $\mu\text{g}/\text{kg}^{\text{a,m,n}}$ Liver (cattle, horses & pigs): 2.5 $\mu\text{g}/\text{kg}^{\text{a,m,n}}$ Milk (cattle): 0.3 $\mu\text{g}/\text{l}^{\text{a,m,n}}$
Tranquillizer		
Azaperone	0-3 ^e	Muscle & fat (pigs): 60 $\mu\text{g}/\text{kg}^{\text{a,n}}$ Liver & kidney (pigs): 100 $\mu\text{g}/\text{kg}^{\text{a,n}}$

NOTES

- a. Expressed as parent drug.
- b. The Committee noted that the concentration of carazolol at the injection site may exceed the ADI, which is based on the acute pharmacological effects of carazolol.
- c. Temporary ADI.
- d. Group temporary ADI for dihydrostreptomycin and streptomycin, either singly or in combination.
- e. Temporary MRL.
- f. Expressed as the sum of dihydrostreptomycin and streptomycin.
- g. The ADI for enrofloxacin is based on antimicrobial activity. MRLs were not recommended because the relationship between total antimicrobial activity and residues of enrofloxacin and ciprofloxacin could not be determined.
- h. Temporary MRLs were recommended because the ADI is temporary.
- i. An ADI could not be established for oxolinic acid because of major reporting and protocol deficiencies in the available studies and because a clear no-observed-effect level (NOEL) could not be identified for anthropany in dogs.
- j. MRLs were not recommended for oxolinic acid because an ADI was not established.
- k. Expressed as the sum of spiramycin and neospiramycin. The MRL in pig muscle and temporary MRLs in pig liver, kidney, and fat are based on total antimicrobially-active residues and expressed as spiramycin equivalents.
- l. The ADI for dexamethasone was established at the forty-second meeting of the Committee.
- m. The MRLs for dexamethasone in cattle and pigs were recommended at the forty-second meeting of the Committee.
- n. Expressed as the sum of azaperone and azaperol.

**RESIDUES OF SOME VETERINARY DRUGS IN ANIMALS AND FOODS
FAO FOOD AND NUTRITION PAPER (FNP) [41/6](#), ROME 1994**

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This document is one of the three publications prepared by the forty-third session of the Joint FAO/WHO Expert Committee on Food Additives (JECFA). The meeting, held in Geneva in November 1994, was dedicated exclusively to the evaluation of veterinary drug residues in food. The report of the meeting will be published in the WHO Technical Report Series, and the toxicological monographs as No. 34 in the WHO Food Additives Series. This document provides information on the chemical identity, properties, use, pharmacokinetics, metabolism, tissue residue depletion and methods for analysis of the substances indicated on the cover. The publication is meant for regulatory authorities, veterinary drug researchers and any other concerned persons who wish to gain information and insights on the needs and problems involved in establishing maximum limits for veterinary drug residues in food.

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